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THE ASPERGILLI

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THE ASPERGILLI

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INTRODUCTION

Species of the great group Aspergillus form a very considerable percentage of all the mold colonies encountered in the cultural examination of foodstuffs, of soil and of miscellaneous materials. Together with the Penicillia and the Mucors, they furnish the "weeds" of the culture room. Certain species of this group have been extensively studied as presenting organisms especially favorable to biochemical experiment. In this way, *A. niger* in one of its forms has become an agent in the commercial manufacture of citric acid by fermentation; other strains of that species are used in the fermentation of oakgalls during the manufacture of pyrogallic acid. Members of the *A. flavus-oryzae* group are widely used in Japan and China in the saccharification of mashes for alcohol fermentation and in the fermentation of soy beans for the manufacture of soy sauce. *A. wentii* was found in a similar industry in Java. *A. okazakii* has furnished a commercial enzyme preparation in Japan; *A. oryzae* is the agent in making Taka diastase in America. *Aspergillus fumigatus* with some of its allies is an important cause of disease in birds, especially in captivity. Many other forms are found to have occasional industrial or pathogenic relations requiring their positive identification.

These illustrations account for the occurrence of a multitude of discussions of Aspergilli as a group and as single species in the researches of botanists, bacteriologists, chemists, physiologists, and medical investigators. No attempt has been made, however, to cite every reference to an Aspergillus found in this mass of literature, but the original description has been found and considered for all species except three for which we were forced to depend upon secondary references. In addition, these names have been followed through the investigations in these widely separate fields, to find either a sound foundation for a permanent nomenclature, or such a basis for identification and description as may simplify the further study of great groups of organisms nearly related but differing so significantly in their activities as to have individually economic, physiological or medical importance apart from the groups to which they belong.

The book is frankly biological and primarily taxonomic. Technically chemical discussions are deliberately omitted, but the biological significance of the numerous biochemical studies of Aspergilli is recognized

by summaries of the results of many such investigations and such citation of the original sources as render the full discussions accessible to the chemical investigator for whom any abstract within the limits of this manuscript would be inadequate.

The beginnings of this study of the genus go back to 1904 when one of us began to investigate the part played by molds in the field of food handling. From the very start, species of Aspergillus were constantly encountered with equally constant embarrassment in their identification from the descriptions available. Correspondence, exchange of cultures, and personal conference with workers in America and in Europe left many of these questions of identification still unanswered. Culture by culture the accumulation of organisms grew, until in self-defense we attacked the taxonomic problem, at first by groups, and finally offer here a reworking of the entire genus as we have encountered it in the literature, in the field and in the culture room.

Many members of various groups have been given a routine microscopic examination without culture, many more have been isolated, their identity to the group recorded and the cultures discarded, others once added to the collection have been lost by some accident or oversight in handling. In some of the groups, the collection was reduced materially by the elimination of what appeared to be morphological duplicates after the publication of previous papers. At present the laboratory culture collection of Aspergilli includes in the various groups the following numbers: *A. glaucus* 39, *A. nidulans* 11, *A. fumigatus* 20, *A. terreus* 8 (reduced from 31), *A. versicolor* 21, *A. ustus* 17, *A. flavipes* 6, *A. sydowi* 9, *A. niger* 44, *A. flavus-oryzae* 90, *A. ochraceus* 20, *A. candidus* 5, *A. clavatus* 8, *A. wentii* and *tamarii* together 40, with a few scattering cultures of organisms of questionable relationship. The present living collection contains, therefore, about 350 strains as a final basis for this publication. The size of the collection varies from time to time, but it will be probably necessary to reduce it, on account of the routine burden of maintenance and to limit the strains kept to representative members of the various sections of each group.

Our thanks for coöperation, material, counsel and assistance of many kinds is due to Dr. Carl Wehmer of Hanover, Prof. Roland Thaxter of Cambridge, to Mrs. Flora W. Patterson and Miss Charles of the Bureau of Plant Industry, the New York Botanical Garden and its mycologists for the use of their collection and library, Dr. C. A. Browne, Dr. E. D. Ball, and Dr. C. L. Alsberg of this Department, Mr. Harold Raistrick of Nobel's Explosives Company, Ayrshire, Scotland, Dr. Kokichi

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In addition, the thoughtfulness and courtesy of the late Dr. Farlow must be especially remembered. Many times during these twenty years, he gave freely the resources of his laboratory, collection, and the treasured literature of his own library for our use besides laying aside for us everything bearing upon our problem, thus contributing of his own time and energy. Finally, whatever of ideals in culture work may have been developed received their original inspiration from weeks spent by courtesy with Dr. Erwin F. Smith in his laboratory many years ago followed through all the years by the privilege of his personal friendship and counsel.

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Bureau of Chemistry

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PART I

GENERAL DISCUSSION

CHAPTER I

Historical Discussion and Generic Characterization

The taxonomic literature of the genus begins with Micheli (1729) (1) and runs through two centuries of descriptive botany, during which about 300 names have been used for specimens mostly as found upon natural substrata and having the gross characters of Micheli's Aspergillus, which were broad enough to include any microscopic fungus with a stalk and spore-bearing head. Micheli was followed in 1742 by Haller (2, 3), who cited Micheli's references to Aspergilli using the same type of descriptive phraseology instead of binomials. He adds (Tomus 1, page 6), however, under "*Aspergillus capitatus ochroleucus*" sufficient descriptive phrases to justify Wilhelm's suggestion that he probably had some member of the *A. ochraceus* group under observation. His "*Aspergillus albus pediculo articulatus*" and "*Aspergillus capite cylindrico-cinereo*" are scarcely sufficient to identify either the white Aspergilli or *A. clavatus*. This is followed by "*Aspergillus ramosissimus in globulos terminatus,*" which evidently brings Sporodinia into his group as is done by many of the later authors. His reference (Tomus 1, page 763) to "*Aspergillus capitatus capitulo pullo seminibus subrotundis*" continues the terms originally used by Micheli for some black organism. Haller repeats in his later publication (1768), as no. 2152, "*Aspergillus capitatus ochroleucos*" with practically the same words as in 1742. He then adds as no. 2153, "*Aspergillus purpureus;*" as 2154, *Aspergillus capitulo pullo*; as 2155, *Aspergillus albus*, etc.; as 2156, *A. "petiolatus"* and 2157, *A. ramosissimus*; in practically the same terms used in 1742, except for the addition of *A. purpureus* and *A. petiolatus* in the later work. This usage apparently connects the application of the term "pulla" for some black headed form by Micheli, with its later use by Persoon and Link. There is more or less continuity in applying the terms "ochroleucus," "albus" and "aureus," but the constant intermixture of Aspergilli with mucors and even myxomycetes continued through a century of descriptive literature and introduced so much uncertainty that few of the forms described before Corda could be recognized, as was noted by Montagne in 1849. Micheli's Aspergilli were cited repeatedly by the botanists who succeeded him with frequent changes in generic name, including especially Byssus, Mucor, Ascophora

and Monilia. Persoon (4) in some of his publications used Aspergillus, in others Monilia for the same form. In citing Persoon subsequent authors have sometimes cited as Aspergillus, species given by Persoon only under Monilia, assuming apparently that Monilia was merely substituted by Persoon for Aspergillus Micheli. Sometimes where the name Aspergillus was followed in the original by a descriptive series of terms, authors have cited the generic name followed by the first descriptive term, as a binomial followed by assigning the binomial to the original author who did not use the binomial system. In spite of diligent scrutiny during the last century, it remains impossible to identify species among these very delicate and perishable organisms in the sense used by any of the authors who preceded Link.

As a basis for classification, generic limitations must first be defined. Restriction of the generic name to its present use is commonly credited to Link (5), who described *A. glaucus*, *A. candidus* and *A. flavus* in terms which have restricted these names to members of fairly tangible series still recognizable. Link (6), however, later included species of Sporodinia among his Aspergilli, thus clearly indicating the purely artificial nature of his grouping.

To define the usage accepted here a generic characterization is proposed.

GENERIC CHARACTERIZATION

Aspergillus

Vegetative mycelium consisting of septate branching hyphae, colorless, bright colored or in a few forms slowly brown in localized submerged areas, or producing brown crusts or sclerotia; conidial apparatus developed as stalks and heads from specialized enlarged, thick-walled hyphal cells (the foot-cells) producing stalks (conidiophores) as branches approximately perpendicular to the long axis of the foot-cells; stalks (conidiophores) unseptate or septate usually enlarging upward and broadening into elliptical, hemispherical, or globose fertile vesicles bearing fertile cells or sterigmata either parallel and clustered in terminal groups or radiating from the entire surface; sterigmata either in one series or as a "primary" series, each bearing a cluster of two to several secondary sterigmata at the apex; conidia varying greatly in color, size, shape and markings, successively cut off from the tips of the sterigmata by cross-walls (not produced by budding), and forming unbranched chains arranged into radiate (globose) heads or packed into columnar

(calyptrate) masses; perithecia found in certain species only, unknown in most species, cleistocarpic, thin-walled, producing asci and ascospores within a few weeks; sclerotia regularly found in some strains, occasionally found in other strains and not found in other and closely related strains, mostly globose or subglobose, commonly composed of thick-walled cells apparently filled with stored material, reported by Brefeld (7) for *A. niger* and by Dangeard as producing asci after a resting period, but such asci and ascospores have not been found by us.

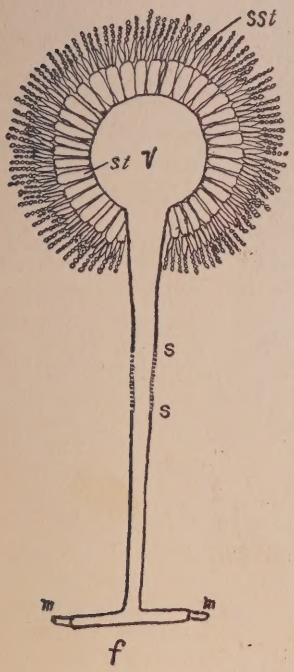


Fig. 1

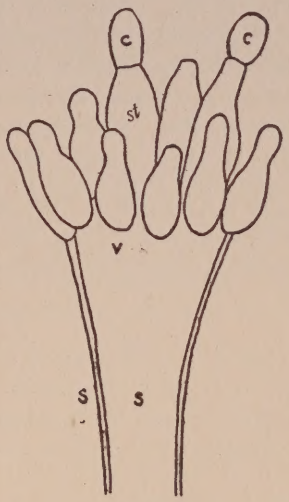


Fig. 2

Fig. 1. Diagram of a Stalk and Head of Aspergillus

f, the foot cell; *m*, vegetative cells at each end of the foot cell; *s*, stalk interrupted since it would be ordinarily much longer; *v*, the vesicle; *st*, primary sterigmata; *sst*, secondary sterigmata with chains or radiating conidia.

Fig. 2. Aspergillus Head with Simple Sterigmata

s, stalk; *v*, vesicular area; *st*, sterigmata; *c*, developing conidium

Our present use of the name Aspergillus for conidial forms with fairly definite morphology is attributable to Corda (8), who redescribed *A. glaucus* Link with notes as to its distribution, but failed to recognize its ascosporic relations. The connection of *Aspergillus glaucus* Link and *Eurotium herbariorum* as conidial and ascosporic phases of the same organism was announced by DeBary (9). The transfer of the species of Aspergillus to the genus Eurotium as made by DeBary following the recognition of the ascogenous genus for one of them, failed of general acceptance, because very few of the aspergilloid organisms known have been found to produce asci.

Eurotium

Parallel with the development of the literature of Aspergillus as a conidial form, we find a series of species under Eurotium as organisms producing closed sessile fruiting bodies whose content of spores was more or less known. Conspicuous among these species were those producing bright yellow to orange perithecia 100 to 300μ in diameter as found on badly dried specimens in phanerogamic herbaria. These were grouped together as *Eurotium herbariorum* attributed to Wiggers and eventually connected with Aspergillus by DeBary (loc. cit.), who proposed to displace the generic name Aspergillus with Eurotium. Few of the Aspergilli have been found to produce perithecia, however, and many prefer to maintain the generic name Aspergillus for the whole group rather than search for nearly related forms in separate genera. This practice is followed by Wehmer (10) and continued here.

Sterigmatocystis

Cramer (11) in studying his black organism (*Sterigmatocystis antacustica*) found in the human ear proposed to separate in the genus Sterigmatocystis, those aspergilloid organisms whose primary series of sterigmata branch so that each sterigma in that series bears a tuft or crown of secondary sterigmata (see fig. 1). The observation that this basis for separation would put into different genera species which are obviously closely related governed Wehmer in discarding Sterigmatocystis. There are sections of the Aspergilli with consistently 1 series of sterigmata and other sections with sterigmata in 2 series, but there are also Aspergilli in which heads of both kinds are found in the same culture and sterigmata of both kinds in the same head. There appears no advantage in maintaining the separate genus. In dealing with the various species discussed our attempt to record the changes in generic

name from Aspergillus to Sterigmatocystis and back again has probably left some bibliographic history unrecorded. It is necessary to trace every species under each name to reach completeness.

Euaspergillus

Ludwig (12) segregated those species of Aspergillus producing sclerotia from those with soft walled yellow perithecia (Eurotium) and proposed the name Euaspergillus for them, designating as species "*E. flavus* (De Bary)," "*E. ochraceus* Wilhelm," "*E. niger* van Tieghem," "*E. nidulans* (Eidam)," "*E. fumigatus Fres.*" and "*E. flavescens.*" In making this separation he assumed that the sclerotia eventually produce ascospores, but presented no data. He left as Aspergillus only those species for which neither perithecia nor sclerotia were discoverable. Although there is some merit in the contention that ascospore production following a sclerotium stage in contrast to the soft walled quickly ripening perithecia of the *A. glaucus* group might be worthy of recognition in nomenclature, his suggestion has not been accepted widely and is not followed here.

Aspergillopsis

Spegazzini (13) proposed to separate the black spored Aspergilli under the name Aspergillopsis, upon the ground that these forms are dematiaceous and should be in a separate genus. No compensating advantage is seen by us in this increase in synonymy. In this group, yellow to brownish color in stalk walls is so unevenly distributed that forms obviously closely related may show walls colorless, walls partially colored, or walls completely yellow or brown. Whatever value brown color as a group character may have among other fungi a separate nomenclature for dematiaceous Aspergilli would be misleading. Sopp (14) about the same time used this generic name for another series apparently near if not identical with *A. ustus* as indicated later in discussing that group.

Diplostephanus

Langeron (15) proposed the name Diplostephanus for perithecial species of Sterigmatocystis, designating *A. nidulans* (Eidam) Winter as the type. He made the further proposal to limit the use of the name Aspergillus to organisms producing conidia only. Under this scheme of nomenclature strains obviously related but failing to produce ascospores would continue in the old genera Aspergillus and Sterigmato-

cystis, and ascosperic forms would go into Eurotium and Diplostephanus. However logical the recommendation may be, it would add seriously to the synonymy without compensating value, hence is rejected here.

In harmony with these considerations, the generic characterization already offered has been developed in terms broad enough to cover the hyphomycetous species commonly included under the form genera Aspergillus, Sterigmatocystis and Aspergillopsis, and at the same time to include those ascomycetous species sometimes segregated as Diplostephanus and that part of those species included in Eurotium, which produce superficial, thin-walled, globose, yellow to orange perithecia. On the basis of examination covering many thousands of cultures, we can see no valid reason for using the name Sterigmatocystis or carrying the name Eurotium for the ascosporic phase of a small number of species obviously closely related to a much greater number which do not produce asci under known conditions.

The information at present available clearly indicates that the group designated Aspergillus by the characterization already offered represents a natural or genetic relationship close enough to be regarded as a single genus for purposes of description and nomenclature.

CLASSIFICATION

In a scheme of classification based upon ascospore formation, Aspergillus or, if preferred, the ascosporic genus Eurotium (or in part Diplostephanus Langeron) is usually classed with the Perisporiaceae (Saccardo, Engler and Prantl, Lindau, Clements, etc.) on account of the structure of the perithecium. Since such a classification fails to account for the great group of Aspergilli whose ascospores are unknown, the names Aspergillus and Sterigmatocystis appear also in Fungi Imperfecti, in the section commonly referred to as the Hyphomycetes, in the order designated by Saccardo as Moniliales, family Moniliaceae, and finally in a subdivision designated by Lindau Aspergilleae where they are contrasted with Harzia, Citromyces, Penicillium, Gliocladium, Amblysporium, Briarea and similar molds.

The basis for generic characterization in this and adjacent groups has been very inadequately studied. Details of spore formation (see page 19) which are of fundamental importance are practically unrecorded in the historic descriptive literature. Too commonly, our knowledge of the species represented under a generic name has been a matter of tradition among groups of workers rather than adequacy of the data available for identification.

CHAPTER II

Morphology

THE ASPERGILLUS COLONY

Since few of the Aspergilli regularly produce perithecia and ascospores' a basis for identifying the majority of the molds of this group as they are actually encountered in nature and in culture must be found in the description of the colonies and of the details of morphology found in the spore-bearing structures available.

The vegetative mass of most Aspergilli consists of submerged mycelium from which only fruiting hyphae rise above the surface. Such colonies suggest a field of ripening grain, in which stalks and ripening heads predominate, and have been described as velvety from their appearance in many species. Some species produce a more or less aerial felt (floccosity) of branching and interlacing hyphae bearing conidiophores as in certain strains of the *A. versicolor* and *A. fumigatus* groups upon culture media; in *A. wentii* under some conditions; and in certain strains of the *A. glaucus* group which form long streamers of hyphae upon meat stored in cool, damp rooms. The character of the surface growth is a diagnostic characteristic which is usually fairly reliable under reasonably uniform conditions of culture.

COLOR

The most striking character of an Aspergillus colony is usually its color production. This takes two general forms: (1) color in the aerial part, hyphae, stalks, and heads (intracellular), as universally included in characterization of species; (2) colors included in the substratum (that is, extracellular), and representing the specific effect of the organism upon particular media.

COLONY COLOR

There is a characteristic range of colors for each group (or collective species) of Aspergilli, with a much narrower range in successive cultures of the particular races or strains. The coloring substance may be deposited in the conidia only, as in *A. flavus;* in the conidial wall, and more

or less present in the sterigmata, vesicle and upper part of the stalk as in *A. niger;* or the heads may be uncolored or nearly so, while the outer layers of the stalk wall may be colored as in *A. flavipes* and *A. ochraceus.* In some species of the *A. glaucus* group, the colony color is at first green with the development of conidia, then predominantly yellow to ferrugineous from ripening perithecia. Again, in other species of the same group, the walls of the stalks and aerial hyphae become encrusted with granules yellow in the young colony and when conditions are acid, but becoming reddish or ferrugineous in age with the change of reaction to alkaline. The colonies then are at first predominantly yellow with green heads inconspicuous on a yellow background and later become rusty red or brown. The granules respond to exposure to acid and alkali as an indicator, yellow in the acid phase, red when alkaline.

In the *A. flavus* group, Saito (see *A. pseudoflavus* Saito, page 205) followed by Thom and Church found that cultures with brighter shades of green when subjected to a vapor of ammonia would lose the green color and assume the somber yellow shades of variant members of the same group, and that this reaction was reversible, since the vapor of acetic acid would restore or even intensify an original green shade. The experiments pointed to the hypothesis that the wide range of shades produced by mixtures of yellow and green in the *A. flavus-oryzae* group may be attributed to racial limitations strain by strain in the range of hydrogen-ion concentration produced by metabolism. When a carbohydrate fermentable by the particular species is present, an acid reaction is promptly produced in the growing colony; as growth progresses alkaline products are also produced. The colony color in this series, therefore, reflects first the intensity of the initial acidity as shown by the intensity of the green color reached. The persistence of this shade or its subsequent reduction or entire disappearance to leave a somber yellow or finally brown colony, represents the balancing of the two activities. As a result, certain strains of this group, if grown on Czapek's solution agar, are quickly and very persistently deep green, others become particular shades of green, which fade to yellow and finally some of them to brown, and a few forms produce no true green color, but assume a somber yellow with the first development of conidia.

Color changes in the conidia have not been satisfactorily worked out for other groups. In the *A. niger* group, the shade of yellow to purple brown or black seems to be a strain or race character little influenced by handling (see Mutations, page 25), which is not destructive of the racial entity. Each race seems to reach a fixed quantitative limit to

the secretion of the coloring substance, thus reducing the most conspicuous diagnostic character among these forms to a quantitative rather than qualitative basis of separation.

COLOR IN CONIDIAL WALLS

The conidial walls may be smooth but carry sufficient coloring matter in diffused form to give the characteristic colony color. Within such series as *A. fumigatus*, *A. nidulans* and *A. ochraceus*, however, strains or races may be found which vary from conidial walls smooth or nearly so to walls bearing echinulations or even traceries apparently produced by aggregation of color substance into spinules, or bars between the outer and inner walls of the cells. Although smoothness and echinulation have received much weight in descriptive literature, in such groups as these, such observations should only be used to separate nearly related strains in the same group, rather than as group characters.

COLORS IN THE SUBSTRATUM

The production of bright colors in the substratum is frequent among the Aspergilli. The color produced by any species or race upon any medium is dependent first on the ability of the mold to elaborate the particular product and second on the presence of the necessary building material in the substratum. A mold may grow well upon a particular medium without discoloring it; a transfer from this colony to another substratum may turn the second medium red or yellow.

Red and yellow colors commonly predominate in cultures of Aspergilli. Enough work has been done to indicate that the yellow colors in most species, at least, are produced in substrata in which growth is accompanied by the presence of acid conditions. Red or purple-red colors are associated with alkaline conditions as far as studied. When color changes occur in the substratum, yellow or orange usually predominates at first and is replaced by red. The red colors when observed are commonly reached through the yellow colors, although the yellow phase may be passed quickly. In the *A. versicolor* group there is every variation from deep yellow media very slowly or only partially turning toward red to strains in which the yellow can not be detected. In addition to forms producing these bright colors, strains not discernibly differing in morphology are found to produce no color in the substratum or only traces of such color.

In the discussion of color production in a series in the *A. glaucus* group, Bainier and Sartory (see *A. disjunctus*, *A. sejunctus*, *A. scheelei*,

A. umbrosus, *A. mollis*, *A. repandus*) emphasized the production of red and yellow colors as diagnostic characters for various species. A few forms in this great group produce yellow colors very persistently upon some media, but if the observation is extended to include hundreds of races the distinctness of this separation into series is obliterated, because a yellow phase can be shown for all of those we have studied and very few of them fail to reach the red phase when observations are continued long enough. Quevedo (16) discussing a member of this same group under the name *A. maydis*, found his cultures to produce a marked fluorescence in the culture fluid and attributed poisoning of stock to the thermo-dynamic effect of the fluorescent products (see also page 84).

Observations of colors produced in the substratum remain, however, very conspicuous and exceedingly useful accessory characters which aid in the placing of species. The describer must bear in mind that such color reactions are confirmative, not absolute characters in separating species.

The final difficulty in dealing with color as a separating character rests in the loose use of color names, which is only partially corrected by the use of color standards (17, 18, 19[1]). Comparison of the same culture by different individuals introduces very considerable discrepancies which become serious when a specific descriptive name or number from one of these standards is introduced into a technical description. In general, a series of observations giving a range of colors for a species is less liable to introduce errors of subsequent identification.

MORPHOLOGY

From the time of Micheli, the name Aspergillus (literally rough head) has been used for molds with a stalk and spore-bearing head.

THE HEAD

The first structure observed in the detailed study of the colony is the spore-bearing head. The color, shape, size, and arrangement of such heads is characteristic of the species. Wehmer (Monogr.) roughly grouped his Aspergilli into Microaspergilli and Macroaspergilli on the basis of the size of the fruiting parts. Thus, *A. fumigatus*, *A. nidulans*, *A. sydowi* and *A. versicolor* would be Microaspergilli and *A. niger*, *A. clavatus*, *A ochraceus*, *A. wentii* and *A. tamarii* would be readily classed as Macroaspergilli. The distinction breaks down when great numbers

[1] The three references most commonly used for fungi.

of forms are studied, but the comparative size of heads and stalks remains a useful adjunct in description as shown in the photographs (plates I and II). The heads in certain species show a consistent range of measurements and form, as in *A. fumigatus* and *A. nidulans* with heads of small diameter forming columnar masses. Similarly the characteristic head of *A. niger*, *A. ochraceus* or *A. wentii* is globose and large. In other species, notably the *A. flavus* group, or *A. candidus*, several sizes and shapes of heads are regularly found in the same colony. The range of size and shape remain, however, characteristic. The observation of many heads in the colony and preferably in many separate cultures forms a better basis for description of sizes and measurements than limited observation. Further description of the structure of head is given in the more logical order, following the discussion of the stalk or conidiophore (page 17).

THE FOOT-CELL

The first step toward conidium formation in this group is the differentiation of certain cells (the foot-cells, fig. 4) in the mycelium for reproductive purposes. These cells become larger, thick-walled, and each usually bears a single conidiophore as a branch, perpendicular to the long axis of the cell and about midway between the ends of the cell. In age these cells frequently become fantastically curved and twisted with their connection to vegetative hyphae inconspicuous but usually still determinable. These foot-cells are commonly submerged in the substratum, although there are a number of strains of the *A. glaucus*, *A. fumigatus*. *A. versicolor*, and especially of the *A. flavus-oryzae* groups in which the conidiophores arise in this way from aerial hyphae. In *A. effusus*, the foot-cells are frequently long and several of them connected together to form whole hyphae bearing considerable numbers of very short conidiophores, hence their differentiation from the sterile or vegetative cells is less easily determined. Failure to recognize the foot-cells as a generic character led Ferdinandsen and Winge (Bot. Tids. 30: 220. 1920) to describe *S. dipus* using the foot-cell as a diagnostic character of the species. The presence or absence of such a differentiated foot-cell is proposed as an arbitrary character to be used in separating certain depauperate forms, which approach the structure and appearance of the monoverticillate Penicillia (Citromyces), from species of Aspergillus. Organisms which lack the typical Aspergillus head with its stalk and especially its foot-cell may be best classified elsewhere.

PLATE I

PHOTOGRAPHS OF ASPERGILLUS HEADS AS THEY DEVELOPED IN PETRI DISH CULTURES AT APPROXIMATELY ONE MAGNIFICATION

2396. *A. niger*, representing a globose head partially splitting at the periphery into columnar masses.

K106. *A. niger*, smaller variety but the same general type.

116. *A. wentii*, a globose head but tending to break up at the margin to show single spore chains or small clusters.

2399. *A. ochraceus*, one variety with large heads globose at first then splitting into two or three divergent columns suggesting sheaves of grain.

112. *A. ochraceus*, another variety with smaller heads splitting in similar manner.

106. *A. candidus*, showing the mixture of large and small heads characteristic of this species.

113. *A. oryzae*, a globose head with periphery fimbriate (individual chains evident).

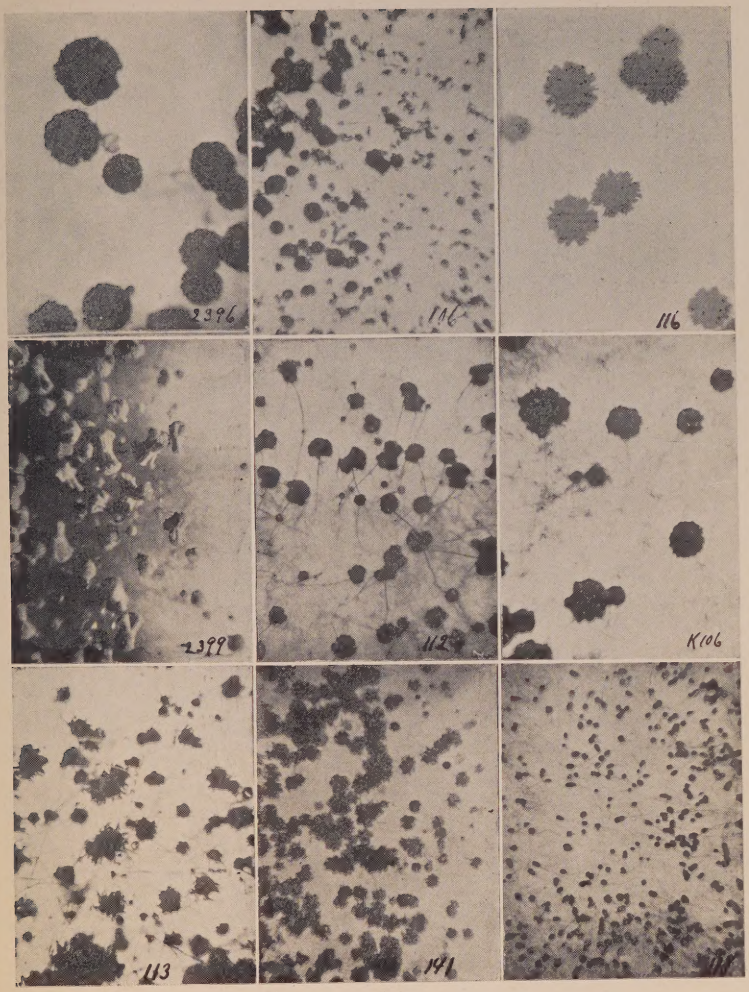
141. *A. flavus* var., denser head but similar to 113.

118. *A. fumigatus*, columnar heads but very much smaller, hence characteristic of the section designated by Wehmer as Microaspergilli.

PLATE II

FIVE TYPES OF HEAD, PHOTOGRAPHED WITH THE SAME MAGNIFICATION

a, *A. clavatus* with long clavate or elliptical fertile area and large stalk; *b*, *A. wentii*, with globose or radiate head and sterigmata radiating from a globose vesicle and with the chains of conidia forming a spherical mass; *c*, *A. ustus*, with hemispherical head in which sterigmata radiate from a hemispherical vesicular area with thin conidial chains; *d*, *A. amstelodami*, a partly columnar, partly spreading head borne upon a stalk composed of thin walled cells shown in partly collapsed condition; *e*, *A. flavipes*, a columnar or calyptrate mass formed by closely adherent chains of conidia.


2396
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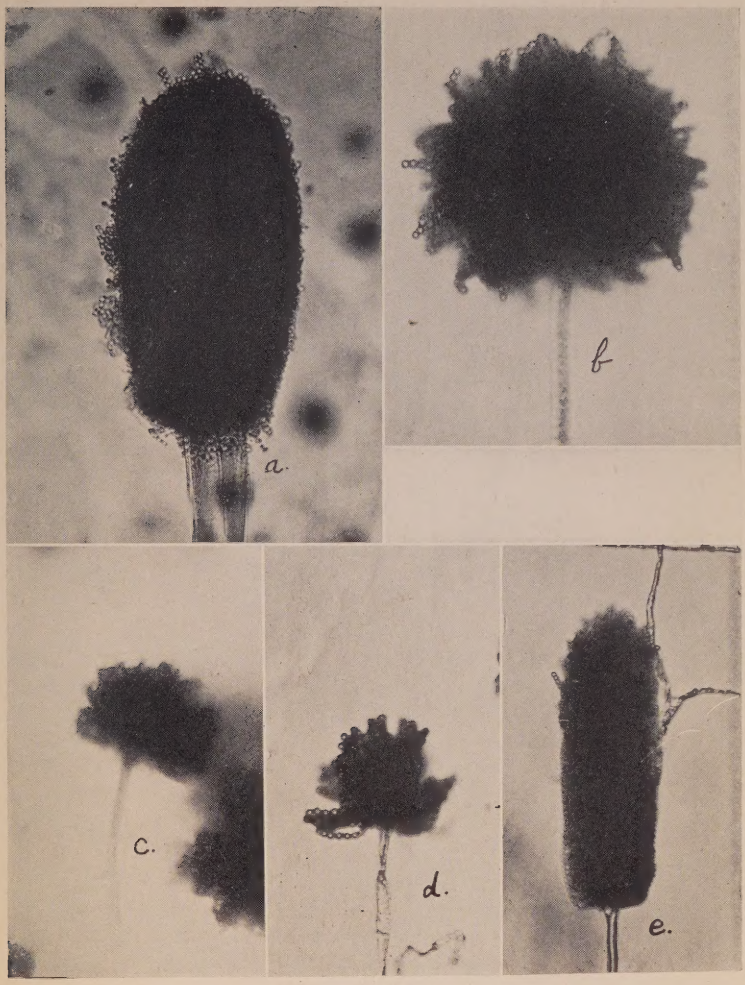

a.
b
c.
d.
e.

THE STALK OR CONIDIOPHORE

The erect, perpendicular branch from the foot-cell constituting the conidiophore usually enlarges upwards toward the apex at which it dilates more or less definitely to form the vesicle. The section of the conidiophore from the foot-cell to the base of the conidial head is designated in this paper as the stalk. The term conidiophore is used in many descriptions as synonymous with the stalk, but since it is frequently used to include both stalk and fruiting head, the term stalk is used here as more definitely designating the support, hence a better usage.

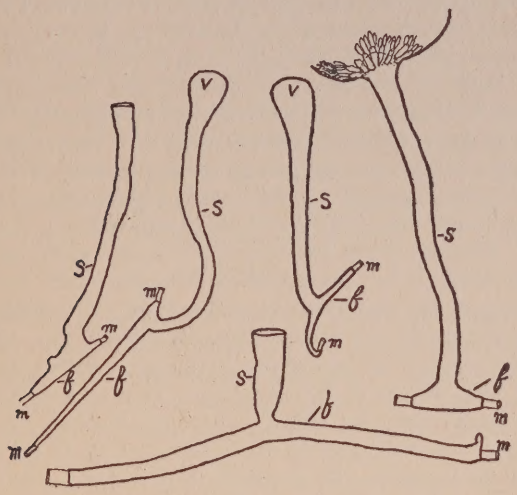


FIG. 4. A SERIES OF FOOT CELLS

s, stalks; *m*, cells of the vegetative mycelium connected with the foot cell, *f*; *v*, vesicle.

The stalk in some groups is not only septate but each cell is sufficiently distinct to justify the term *articulate* which is frequently encountered in the older descriptions. In our experience in examining specimens, *articulate* stalks are found only in *A. glaucus* group. In most Aspergilli the unity of the whole stalk is fairly accentuated. Septa, if present, are thin, fragile and inconspicuous, while the whole stalk is enclosed by characteristically thickened walls without conspicuous nodes as an evidence of septation.

Thickening of the stalk wall may be uniform, or may be greater at the base, thinning to negligible toward the apex. Two general sections based upon the character of the thickening of the stalk wall are fairly readily distinguished, although the careful use of high magnification is occasionally necessary to separate certain strains. In the first, the outer surface of the wall is free from pits, warts or roughenings, hence is called smooth; its structure is difficult to identify; the mass of the cell-wall appears homogeneous or nearly so under the microscope and does not absorb the ordinary protoplasmic stains. When broken many of these stalks show uneven or jagged ends like a broken glass tube; in the *A. niger* group the broken ends split like bundles of lath, giving a possible clue to the method of their formation. In the second section, the wall appears dotted or pitted, or as interpreted with low magnification often rough or echinulate. The secondary thickenings in such stalks appear to have been laid down around protoplasmic areas, which for a time, at least, maintain contact with the primary wall outside. The size and abundance of the pits in the mature stalk wall differ with the species, but in a general way correspond with the rate of withdrawal of the protoplasmic mass from its primitive connection with the original outer wall. In some of the species in both groups, warts, or superficial and usually more or less hemispherical concretions are found on the outer surfaces of the stalk wall sometimes few and scattered widely, again fairly numerous, but always unevenly distributed. These warts or concretions appear to be deposits of excreted substances, possibly due to the evaporation of the numerous drops or globules of liquid abundantly visible upon the young and growing stalks.

The color of the stalk wall may be homogeneous, or the layers may differ markedly in shade. In a number of the groups the entire wall is hyaline. In certain other groups the outer layer is yellow as in *A. ochraceus* and *A. flavipes*, or some shade of green, brown or avellaneous. In some species the whole wall is colored for all or part of its length. No explanation of these color differences is available, except possibly the varying concentration of aspergilline (Linossier (20)) in the upper part of the stalks of members of the *A. niger* group as well as in the conidial heads.

COREMIA

Coremia in the broader sense of ropes of hyphae anastomosing and trailing upon or near the surface of a substratum occur more or less commonly in Aspergilli of certain groups; as specialized erect aggrega-

tions of fruiting stalks (Stilbum-like), such structures are described and figured only for *A. vitellina* by Ridley (page 187), but since Ridley apparently described his form only as collected upon a natural substratum the actual development of a specialized structure remains doubtful. Ridley's figure could be repeated many times by roughly drawing masses of stalks bursting through the otherwise unbroken surface of a rich nutrient substratum such as seeds of cereals, producing a dense cluster of conidiophores.

THE VESICLE

The stalk is usually much larger toward the apex than at the point of origin. At the base of the head a further dilation occurs more or less abruptly to produce the vesicle (*Blase* of the German mycologists). This vesicle is globose, hemispherical, elliptical or long clavate in various groups of Aspergilli and furnishes an enlarged surface for the attachment of spore-bearing cells. The lumen of the vesicle is continuous with that of the upper part of the stalk; a septum near the base of the head is occasionally but only rarely seen (see Corda, *A. mucoroides* for description), but has not been regularly found in any species.

STERIGMATA

Part or all of the surface of the vesicle is covered by a layer or series of cells directed upward if clustered on the apex only, or radiating from the whole surface where the vesicle is globose. These cells either bear conidia directly or each bears a verticil, cluster, or crown of cells forming a secondary and usually closely packed layer. These series are variously termed, sterigmata (singular sterigma) or basidia if in a single series; if in two series those next the vesicle are called primary sterigmata or by some authors basidia and the outer series secondary sterigmata; when the term basidia is used for the primary series the term sterigmata alone is used for the secondary series. Following Wehmer (10), the terms primary and secondary sterigmata are used in this work (see figs. 1 and 2).

The primary sterigmata form a series of similar cells usually closely packed over the vesicular area, but retaining intimate connection with the protoplasm of the vesicle. This is conspicuously seen in certain species with walls of the vesicle much thickened and showing a definite pore at the base of each sterigma. The secondary sterigmata when present have a general similarity throughout the group as conidia-bearing cells with morphology characteristic of the genus, not distinctive

of the species. On the other hand, the primary sterigmata, whether directly conidia-bearing or not, by their size and arrangement have usually a distinct group significance within the genus. Their length and diameter, and the proportion of these measurements to that of the vesicle, together with their arrangement on the vesicular surface, are

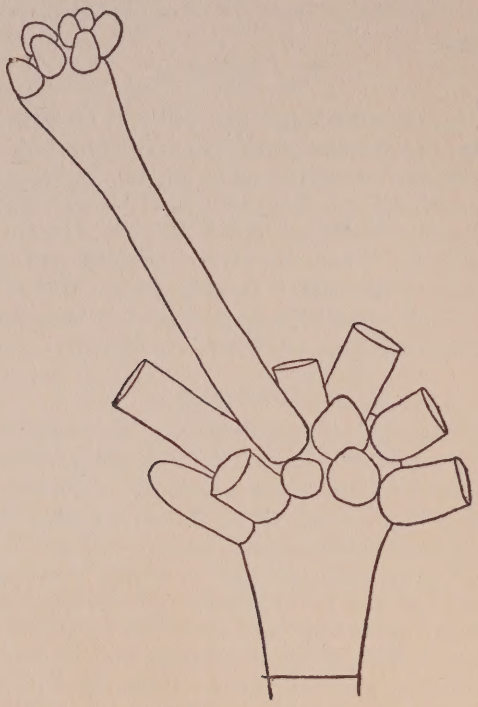


FIG. 5. VESICLE AND STERIGMATA OF A. GLAUCUS VAR. SHOWING ONE STERIGMA GROWN INTO A SHORT STALK BEARING A SECONDARY HEAD

Several such secondary stalks and heads were developed in this same group

group or even specific characters which should be carefully noted for each specimen examined. Septation of primary sterigmata is reported for certain species and is occasionally seen in culture, but has not been found characteristic by us for any species. Proliferation of sterigmata to form sterile hyphae abundantly surrounding a head has been described

for *S. auricoma* by Guéguen (page 187); occasional proliferation of individual sterigmata is very common in many species. Proliferation to produce one to many short secondary stalks bearing little heads is very common in certain strains of the *A. glaucus* group (fig. 5) and occasionally even in *A. niger* and, perhaps, other species.

CONIDIA

The conidium producing organ in Aspergillus is a specialized cell (*sterigma* of either the primary or of the secondary series), forming a structure characteristic of a group of related genera, including Aspergillus, Penicillium, Gliocladium and, perhaps, others. The specialized cell narrows at the apex in a manner characteristic for the species or

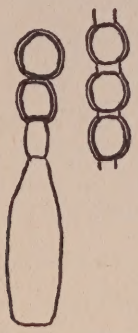


FIG. 6. DIAGRAM OF STERIGMA AND CONIDIUM PRODUCTION

group, into a thin-walled tube from which all conidia arise. The tube lengthens, cell division takes place followed by the formation of a secondary wall by the newly formed conidium within the original wall of the tube. This is followed by rapid growth and assumption of the size and shape characteristic of the species. Meanwhile the tube continues to lengthen below the new conidium and repeats the process for each conidium. An unbranched chain of conidia is, therefore, produced (fig. 6).

The original conidial wall may remain visible as a "connective" "disjunctor" or "bridge" between the conidia in the chain and may be further emphasized by the deposit of granules, tubercles or bars of color between the outer or primary wall and the inner, secondary or true conidial wall, as in *A. niger*, *A. tamarii*, or *A. citrisporus*, or by the

formation of wrinkles or spinulosities apparently involving only the outer wall as in *A. nidulans*, *A. versicolor* or some strains of *A. ochraceus*. In other species, the outer and inner walls are indistinguishable, even though the inner wall may be much thickened, pitted or grooved as in some strains of *A. flavus*, or in most members of the *A. glaucus* group; in these pitted conidia the primary wall appears in some species to dip down into the depressions or pits, leaving the surface actually roughened or to bridge the pits which show beneath it. In conidia of *A. niger*, we found it possible to dissolve the color bars in warm water, after which the primary wall was visible as a loose, thin, wrinkled envelope, surrounding the firm, smooth secondary wall of the conidium. This method of conidium formation was discussed by Thom (21) for Penicillium in 1914, but has been called endogenous by Sartory and Sydow (22) in describing Aspergilli.

In contrast to the cutting of new cells from the tip of a spore-bearing tube which restricts the resulting conidia to an unbranched chain with the newer cells always at the base, the conidia in such species as Cladosporium and Hormodendrum begin to bud out from the tip of a more or less specialized erect or prostrate branch and continue to bud from the apex and from various nodes of the chain to produce bushlike or treelike masses with the newer cells always at the tips of the branching system.

CYTOLOGY OF CONIDIUM FORMATION

Dangeard (23) in his cytological study of the origin of the perithecium in the ascomycetes discusses conidium-formation also in a series of Aspergilli. In *Eurotium herbariorum* he finds sterigmata cutting off multinucleate conidia. From his figures it would appear that the nuclei migrate directly from the vesicle through the pores in the wall into the sterigmata, although the statement is not directly made. His figures of developing conidia distinctly show more than one nucleus. This species (probably representing a group condition) is in direct contrast to all of the other species of Aspergillus figured and described by him. *Aspergillus flavus* Link is described and its sterigmata and conidia figured as mononucleate. Similar figures are given for *Aspergillus fumigatus*; in *S. ochracea* and *S. nidulans*, the double sterigmata are still mononucleate. In *Aspergillus niger* he figures and describes double sterigmata, each with a single nucleus; we also found the conidia and sterigmata of *A. niger* (no. 111) to be mononucleate. The drawings of Dangeard are, however, so obviously schematic that aside from representing his observations of nuclei, they are not representative of the

morphology of the parts involved in spore-production. In his figures he has entirely failed to take into account the cytology of the foot-cell of the spore-bearing stalk, which is the characteristic organ of all these species. His figures of sterigmata are distinctly misleading in failing to show the actual shape of the cells found. If Dangeard, however, is correct in reporting the cells in the conidial apparatus in the *A. glaucus* group as multinucleate, it forms a distinct line of separation of this group from the remainder of the great series of Aspergilli.

PERITHECIA

The general morphology of the perithecium of the *A. glaucus* group (Eurotium) was described by De Bary (24), and De Bary and Woronin (25) for the *A. glaucus* group and by Eidam for *A. nidulans* in 1879. The description of De Bary fixes the type of yellow to orange or ferrugineous perithecium from 90 to 300μ in diameter, without ostiole, and without specialized appendages, as found in most of the species of the *A. glaucus* group. These perithecia are borne above the surface of the substratum and are never more than loosely hung in networks of hyphae; they are often very abundant and dominate the color of the colony. Perithecium formation is favored by abundance of assimilable carbohydrate, but may or may not be completely suppressed by its replacement with nitrogenous products; transfers from the same culture have developed colonies yellow from perithecia when grown on sugar solution or dense green without sign of perithecia upon a peptone medium or a piece of leather.

Mature perithecia are described for *A. nidulans* by Eidam (26) but without giving attention to their origin. In this description, Eidam described a loose network of hyphae more or less completely surrounding the perithecium containing large numbers of "Hülle" cells (fig. 7), terminal or intercalary cells which swell and become vesiculose, elliptical or almost globose, then develop very heavy thick walls almost obliterating the cell lumen. These cells were later noted by Dangeard (loc. cit.), who designates them as chlamydospores, but without presenting evidence of function as propagative cells. We have found these cells abundantly in connection with perithecia in all strains of the *A. nidulans* group, and cells of the same general character in sterile masses of hyphae in some of the strains of the *A. terreus*, *A. flavipes*, and *A. ustus* groups.

The perithecium of Aspergillus arises from a branch which coils in various manners in the different species to become the ascogone, as figured by Dangeard from forms reported as *E. herbariorum*, *A. flavus*,

A. fumigatus, S. ochracea, S. nidulans, by Fraser and Chambers (27) for *A. herbariorum* and by Dale (28) for *A. repens.* No fertilization process was demonstrated in those studies. Dangeard reported the cells in the fruiting apparatus as multinucleate in *E. herbariorum,* but to be mononucleate in the other species figured. In general, however, the development of an ascogone followed by the development of the perithecial wall and accessory cell masses from vegetative hyphae below the ascogone has been roughly described and figured for the group represented by *A. glaucus* and *A. nidulans,* but interpretation of the other specific names used by Dangeard is doubtful, even for grouping, on the basis of the descriptions and figures given.

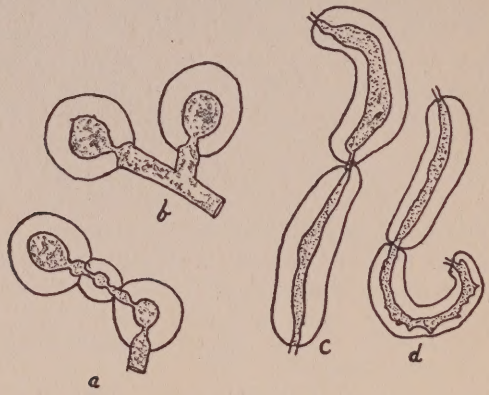


FIG. 7. THE SO-CALLED HÜLLE CELLS

a, b from *A. nidulans; c, d* from *A. insuetus.* These cells are terminal and in chains and massed together looking like tangled chains of sausages. As they ripen the walls thicken greatly and frequently unevenly, while the cells become twisted into shapes more or less characteristic of the species; s-shaped, helicoid, globose, and sinuate forms are sometimes found in the same preparation. The significance of these cells in the life history of the mold is still unknown.

THE ASCOSPORE

So far as fully described the ascospore of Aspergillus follows the type shown in figures and description of De Bary (loc. cit., tableXI, figs. 27 to 30). In the course of its development, the secondary thickening of the cell-wall develops in the form of two symmetrical valves suggesting the arrangement found in the shell of a bivalve mollusk, such as

the hard clam, (*Venus mercenaria*). The ripe ascospore is commonly shaped as a double convex lens with the valves more or less closely in contact at the edges. A series of variations is, therefore, possible as suggested in the diagrams shown in figure 8. If the exospore is in close contact at all points and the valves are brought together figure *a* is produced; a loose fold of exospore at the line contact of the valves gives figure *b*, which complies with description given by Grijns for the ascospore of *A. fumigatus* (*A. pseudo-nidulans* (Grijns) Vuillemin); if the valves remain slightly separated and a fold of exospore develops at each margin figures *c* and *d* are produced as in the *A. herbariorum* series; if the exospore is further thrown into folds or wrinkles on the surface of the valves figure *e* develops as is found in *A. echinulatus*, *A. nidulans*

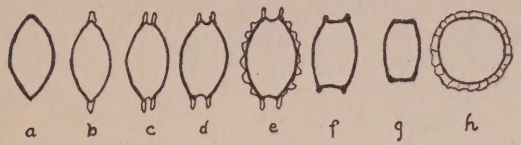


FIG. 8. DIAGRAMS OF THE ASCOSPORE OF ASPERGILLUS

a to *g*, optical section, viewed from the edge of the disklike spore; *a*, ascospore without frill or furrow, *b*, with single frill at the line of union of the valves as suggested by Grijns' description for *A. fumigatus; c*, valves slightly separated with trace of furrow and two frills; *d*, valves definitely separate, furrow evident and frills broad as in the series of *A. herbariorum; e*, furrow and frills evident with exospore thrown into folds or wrinkles on the convex faces of the valves as in *A. echinulatus*, *A. nidulans* and *A. fischeri; f*, valves further separated, frills reduced to traces only, and furrow scarcely visible; *g*, neither frill nor furrow as in *A. repens; h*, lenslike ascospore viewed from the convex surface of the valve showing the frill extending beyond the edge of the valve. All of these forms except *a* and *b* can be readily found with many intermediates bridging all gaps in such a series.

and *A. fischeri*; if the folds are reduced or absent figures such as *f* and *g* appear as in the series related to *A. repens*.

When such an ascospore germinates the figure shown by De Bary (24, fig. 30) develops. Exactly the same form of germination is shown by *A. nidulans* (fig. 9), in which as the spore swells, the valves first separate at one edge, then parting completely remain on opposite sides of the germinated spore conspicuously identifiable by the bright purple red color of the valves.

In certain species described as ascosporic single measurements and descriptive terms are given without either figures or detailed descriptions, as in *A. belfanti*, *A. malignus* and *A. fumigatoides*.

SCLEROTIA

Definite hard masses with characteristic surface marking and coloration, and consisting of thick-walled parenchyma-like cells occur in several groups of Aspergilli. Such structures have not been seen in the groups typified by *A. clavatus*, *A. glaucus*, *A. fumigatus*, *A. nidulans*, or *A. terreus*; they develop regularly in certain members and occasionally under undefined conditions in members to the *A. candidus*, *A. niger*, *A. ochraceus*, *A. tamarii* and in the *A. flavus-oryzae* groups; in other members of these groups grown under similar conditions, no such structures have been found. Their development has not been followed in sufficient detail to fix their genetic significance, but the specialized, characteristic structure of the sclerotia of many of these species lends color to the report of ascospore formation by Brefeld (29) and Dangeard (23), although we have not been able in ordinary microscopic examinations made without serial sections to confirm such development.

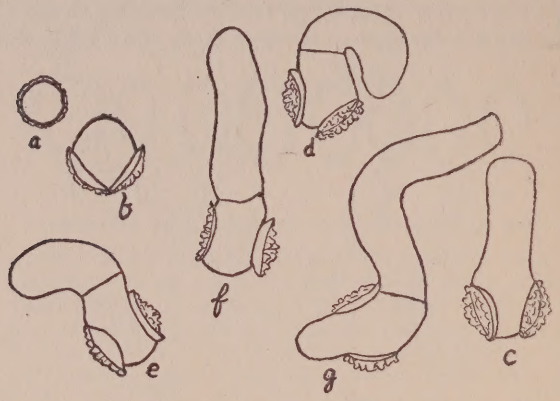


FIG. 9. GERMINATION OF THE ASCOSPORES OF A. NIDULANS

a, the ripe ascospore; *b*, valves separating by swelling of the spore; *c*, to *g*, various forms of germinated spore in which the bright purple red valves of the ascospore with their almost colorless frills and ridges are variously shown still attached or partially peeling away from the cell-wall of the germinating spore.

DEGENERATIONS

Schramm (30) described a form which he believed to be a degenerate condition of *A. niger*. This culture was also studied by Wehmer (31),

who found the organism to be without the power of producing oxalic acid from sugar and who sent the culture to us after suggesting in his paper that a contaminating form had probably displaced the original *A. niger*. Comparison of this description with many cultural studies leads us also to doubt whether the degenerate appearance of this culture was due to physiological degeneration or to partial or complete replacement by some contaminating organism. Some strains do lose many of their characters after many transfers in media which are unfavorable. Interpretation of the real meaning of "unfavorable" presents many problems. Recent studies of nutrition with their emphasis upon "vitamins" and upon quantities of various elements, so small as to defy determinations by the ordinary analytical methods, present the probability that much of our work upon the nutrition of molds must be repeated with much greater precautions before we can generalize with safety as to the exact physiological needs of Aspergilli.

Certain species respond to environment by very conspicuous changes in structure. Two strains of *A. fischeri* which produce perithecia regularly almost without conidia at temperatures of 22 to 30°C. produce abundant conidial heads forming typical velvety green colonies at 37°C., and again at temperatures below 20°C. *A. clavatus* grown continuously on Czapek's solution agar becomes dwarfed to almost unrecognizable proportions.

MUTATION AND VARIATIONS

The collector of Aspergilli who goes further than a few cultures in each of the great color groups (*A. niger*, *A. ochraceus*, etc.) quickly loses his confidence in his ability to describe the particular organism before him in culture, in morphological terms sufficiently definite to insure his own recognition of that strain some years later. Yet the experience of those of us who have kept particular strains in culture longest leads to the belief that many Aspergilli, if kept in culture under favorable conditions, will maintain the same morphological and physiological reactions through many generations. Currie after keeping 3528.7 (*A. niger*) in culture for ten years reports (personal communication) that its vigor and dependability in the production of citric acid from sucrose has not changed. He finds it necessary, however, to maintain his stock cultures with great care, plate out and examine them frequently and use for propagation only vigorous characteristic colonies. He believes this to be primarily a precaution against contamination. Many cultures in our collection are apparently unchanged after ten or

some even after twenty years. Other cultures maintained upon the same substrata lose vigor, become dwarfed, and fail to return to their former appearance in the experiments so far performed; such a result has been interpreted as indicating deficiency in the nutrient substratum rather than failure in the organism. On the other hand, a number of workers, Schiemann, Waterman, Haenicke, Thom and Church, report the actual formation of mutants from Aspergilli, mostly when cultures were definitely stimulated by particular reagents which were more or less poisonous. These mutants appeared in Schiemann's (32) work as isolated heads and groups of heads much paler in color than the ordinary *A. niger*, or in very long stalked heads. Transfers from these heads were regarded by her as true mutants. Of three mutants described by her, we have maintained *A. schiemanni* (*A. fuscus*) and *A. cinnamomeus* since 1914 without apparent change, but did not find *A. niger* var. *altipes* to differ in any marked manner from other long stalked black Aspergilli.

No cytological study was made by Schiemann. Waterman (33) induced the formation of mutants by cultivating *A. niger* in media containing such reagents as boric acid, copper sulphate, para-oxybenzoic acid, salicylic acid, galactose, rhamnose and tartaric acid. In general, products which restrict mold growth were favorable to the tendency to form mutants.

Haenicke (34) using cytological methods found certain forms produced as a result of similar stimulation and showing conidia two to three times the usual size for *A. niger*. These large conidia, apparently with the appearance of Bainier's *S. fusca* and *S. carbonaria*, were multinucleate in contrast to the mononucleate condition of the usual type. Thom and Church (36) after maintaining a culture identified by them as *A. effusus* Tiraboschi in culture many years, apparently obtained a closely similar organism from one of the common types of *A. flavus*. They also found it possible by proper selection to secure the usual type of *A. flavus* by selected transfers from the colony of *A. effusus*. This has recently been repeated by Carlton Burnside (personal communication). Such experiences justify the conclusion that the *A. flavus* group is capable of great variation under more or less definable culture conditions.

In a recent paper, Blochwitz (35) working with *A. versicolor* and using comparative data from other species, reports observations of the variations in the shades of color in a series of strains in the *A. versicolor* and *A. glaucus* groups especially, and in particular strains under different conditions of culture. He reports that when cultures were stored

nine years certain components of the colony color were partly or completely suppressed so that forms normally green produced some subcolonies pure white, while in others the bluish color was intensified. Several series of observations of the same kind are offered.

While generalizations on the subject of color mutations are, perhaps, dangerous, such a study as that of Blochwitz follows the same line as Schiemann and others in indicating that certain of these groups occasionally produce conspicuous variations in response to major stimuli. Not only so but there is great variability in the responses of a particular strain to environment even when the conditions of growth are favorable. Nevertheless, we have found it possible to maintain strains of Aspergilli in culture for long periods without apparent change. On the other hand, the mutation experiments cited must be considered together with the great variety of strains occurring in nature and differing in only a quantitative manner over long periods of varied treatment in culture. Such strains show differences in the intensity of color due to the volume of coloring matter produced, to variability in its deposit in the sections of the stalk or in the elements of the fruiting head or, perhaps, due to the entire suppression of some element in the color complex, or again they may differ in the measurement of elements of the fruiting branch, or in tolerance to inhibiting substances or in quantitative ability to produce a particular enzymic activity. These contrasting experiences support the hypothesis that a great variety of forms with common group characters, but maintaining minor differences indefinitely when grown under favorable conditions may be a response of an unstable or mutating group to stimulation.

GENETIC GROUPS

In Aspergillus, as previously in Penicillium (36), great groups came to be recognized early in this investigation. Species within such groups have certain essential morphological characters in common and usually present biochemical characters fundamentally related in kind, but frequently varying greatly in intensity. Within these groups, large numbers of strains were encountered among which occasionally one would be found to agree with a published description. In addition, cultures were found at times which diverged rather widely from these larger groups.

GROUP RELATIONSHIPS

Such considerations as those proposed in Blochwitz's paper, lead directly to a speculative consideration of the possible origin of these

groups of races or species which constitute the genus Aspergillus as we have it to-day. As presented in the taxonomic sections of this book, a large majority of the strains encountered are readily placed in about 13 groups within which the evidences of relationship are readily seen. Necessarily the characterization of such groups is more or less arbitrary. There remain occasional forms which occupy intermediate or indeterminate positions. These either lack some element defined in group characterizations or present some variant and unexplained character, hence for lack of adequate knowledge must be placed arbitrarily in any scheme of arranging the species within the genus.

Study of the whole basis of grouping, however, presents some suggestions which may be useful in the study of groups or individual species. The authors have placed the *A. glaucus* group at one end of their classification scheme and the *A. flavus-oryzae* group at the other in contrast to Mangin (37), who indicates that separation of these two series is difficult. This separation was based upon the observation that the secondary thickenings of the stalk-wall when present in *A. glaucus*, were apparently homogenous, leaving a smooth instead of a pitted surface and that this character served to separate two whole series of groups. The further contrasting character is the presence of a soft walled quickly ripening perithecium (Eurotium type) in *A. glaucus*, but no sclerotia, whereas in the *A. flavus-oryzae* group sclerotia are common throughout and perithecia of the Eurotium type are not found. The only common bonds between these two great groups are the presence of green color in most of their conidia, the pitting (echinulation ?) of the conidial wall, and the size of the conidia themselves. These similarities become less important under close scrutiny, however. The markings of the walls differ materially in nature when examined with high magnifications, while the ranges of color and size are not really significant in comparison with other morphological characters.

If then, our generic study be made to begin with the *A. glaucus* (or Eurotium) group, examination of the intermediate series should disclose connecting forms. This is exactly the case. Forms like *A. penicilloides* Spegazzini, *A. gracilis* Bainier, and *A. conicus* Blochwitz have conidium producing apparatus with the morphology of the *A. glaucus* group even in the markings of the conidial wall of certain strains, but point directly toward the Microaspergilli (*A. fumigatus*, *A. nidulans*, *A. versicolor*) in their spores less than 4μ in long axis, globose in some species, with conidial chains massed into columns in others and again remaining with the radiate arrangement. Whether adherence of chains in closely

packed columns is indicative of an adhesive substance in the conidial wall, which when excessively developed gives the slimy masses of *A. conicus*, has not been settled. Careful study of this intermediate series thus points to a closer relationship between the *A. glaucus* type of structure and *A. fumigatus* than to the *A. flavus*-like forms.

Among the Microaspergilli (*A. fumigatus*, *A. nidulans*, *A. terreus*, *A. ustus*, *A. flavipes*, and *A. versicolor*), there are the sharp contrasts of color and doubling of series of sterigmata which are used arbitrarily for separation. The single series of sterigmata rigorously maintained in *A. fumigatus* and its close allies points directly through such intermediate forms as *A. gracilis* Bainier toward *A. glaucus*. In *A. nidulans* and *A. sydowi*, however, uniformity disappears. Variations in the arrangement of sterigmata run all the way from the strictly Sterigmatocystis type of heads with two series of sterigmata, through simplifications to single sterigmata with single conidial chains, borne upon aerial hyphae. Some of these heads approach the biverticillate arrangement of certain Penicillia and apparently were used by Sopp (14) in describing his genus Aspergillopsis in his monograph of the Penicillia. The obvious relationship of such groups as *A. fumigatus* and *A. nidulans* is, however, ample reason for discarding Sterigmatocystis as a generic name. In this series of Microaspergilli, the colors of the various species contain elements giving bluish, green, yellow (including brown) and avellaneous tones to the resulting colonies. Yellow, orange, red and purple red colors appear in the substratum, frequently in the submerged mycelium, and may probably affect the colony color also. Cultures of any Aspergillus followed throughout their growing period and held until all activity ceases show changes in color peculiar to the race or strain and within a range of tints and shades characteristic for the species or aggregate species to which the race belongs. In most cases, the color scheme of the colony observed throughout its active period will show, at least, two and often more color elements.

Of the Microaspergilli, *A. fumigatus*, *A. nidulans*, *A. versicolor*, and *A. sydowi* contain a green color factor. In contrast, *A. terreus* lacks green color entirely. Its conidial morphology is otherwise very nearly that of *A. nidulans*. Blochwitz, Schiemann and the authors have pointed out the occurrence of color changes in such organisms by intensification, reduction, or even suppression of the secretion of the coloring substance. For example, *A. cinnamomeus* Schiemann derived under known conditions from a typical strain of *A. niger* is excluded from the *A. niger* group in a scheme of classification based upon color and its

distribution. Similarly, *A. terreus* may have been derived from the same source as *A. nidulans* by the suppression of green color and intensification of the avellaneous coloring elements more or less discernible in cultures of *A. nidulans* and several of the related series. The further suppression of the avellaneous color in the conidia, leaving it weakly present in the stalk, would produce the white columnar heads and yellowish stalks of *A. flavipes*. Again intensification of the coloring element or elements leading toward yellowish brown to reddish brown would produce the brown colonies of *A. ustus* or the still darker *A. insuetus*. These species, while arbitrarily separable by color, may readily be conceived to have close relationship as is further indicated for all of them by the regular or occasional development of clusters of thick-walled sterile cells (the "Hülle" cells of Eidam) even without the production of perithecia.

The *A. versicolor* and *A. sydowi* group with green, blue-green and globose (or radiate) heads, in which green colors are occasionally suppressed, may possibly be linked with *A. glaucus* through such intermediates as *A. penicilloides*. Again where the green color is entirely suppressed they approach the *A. candidus* group, whose colonies while typically white often show yellow tones in age. The white Aspergilli, however, with their colorless stalks and their larger globose heads show many strains differing from the black Aspergilli (*A. niger*) only in the absence of coloring substance. Cultures of white Aspergilli have been grown many times whose detailed morphology was that of *A. niger*, if freed entirely from its characteristic color. Again among the variant forms apparently related to the *A. candidus* group some are found with stalk walls not completely smooth; the suggestion of pitting appears in one strain received as *A. okazakii*, for example. Certain yellow sclerotial forms show walls with only the barest suggestion of pitting. Such observations suggest the bridging of the gap between smooth stalked and pitted stalked sections of the genus.

The groups showing stalks with pitted walls partly lack green color; *A. ochraceus* and *A. tamarii* with their allies and connecting forms are yellow to brown. Every gradation is shown among them from ochraceous or yellow forms with double series of sterigmata and very small smooth conidia, through a connecting series with conidia increasing in size, showing first spinulose points, then echinulations, then a tracery of color bars, all in yellow or ochraceous tones, to forms with large conidia rough with brown color bars and with the secondary sterigmata partially suppressed. As indicated in the Key (page 221), variant types appear

to indicate that the suppression of the brown color and its replacement by green gives the final transition from this group to the *A. flavus-oryzae* group with yellow green colors, heads with double series of sterigmata, mixed series, and heads with one series of sterigmata only.

In presenting so complex a genus, any attempt to trace the relationship or connection of groups of species to other groups within the genus offers many points for disagreement. The data presented are believed to be sufficient to justify the contention that the organisms here brought together as Aspergilli actually belong together, not in separate genera under a series of names.

CHAPTER III

Basis of Description and Classification

INTERPRETATION OF DESCRIPTIONS

In interpreting the descriptions of Aspergilli in the literature certain assumptions, although not always justified, form a working hypothesis for presumptive identification. Stalk (conidiophore) walls and conidial walls are assumed to be colorless and smooth, unless color or markings are either figured or described. Colors are assumed to apply to the general color scheme of the colony, unless specifically applied to the conidia, ascospores or other details by the describer. The color of the conidial head has been made a basis for separation in spite of the difficulties involved in interpreting descriptions. These difficulties are less for the worker with a growing culture before him than for the one handling descriptive literature alone, since the presence of white, green, gray-green, brown or black heads is readily distinguished with a handlens, even though sparingly produced upon a colony with another color predominating as in many members of the *A. glaucus* group or in *A. flavipes*.

EXTENT OF STUDY

Interpretation of descriptive literature accompanied and supplemented rather than preceded the study of great numbers of cultures so that the groups established and the tentative placing of forms not studied in culture are based upon the actual handling of thousands of cultures representing hundreds of strains of Aspergillus handled during a period of more than twenty years. Many of these were studied on natural substrata before their isolation. In addition, examination of exsiccati from several large herbaria, while more or less unsatisfactory as to detail in identification of species, furnished confirmatory evidence of the soundness of the groupings proposed.

A TAXONOMIC KEY COMBINING LITERATURE AND CULTURAL EXPERIENCE PROPOSED

Careful comparison of descriptive notes from many hundreds of cultures showed the desirability of bringing together all that could be veri-

fied from the literature, from exsiccati, and from culture into a taxonomic outline or Key into which new material could be fitted as it was developed. Such keys are, therefore, proposed (pages 93 and 221). An effort has been made to give, at least, a tentative place in this key to every described species for which sufficient information can be gleaned from the diagnosis, from figures given, or from the discursive notes of the original describer and his co-workers. Very many of the descriptions were found inadequate for positive identification of particular strains, but sufficiently definite to indicate group relationships very clearly. Other descriptions contain only enough hints as to group relationship to justify a tentative placing of the species in one of the larger sections. Occasional descriptions leave the species entirely unrecognizable.

STRUCTURE OF THE PROPOSED KEY

In bringing all this material together into a Key, care has been taken to show what groups represent materials actually handled in the laboratory. For that purpose cultures are cited by number (italics) in juxtaposition to the names accepted, or where identification is not reasonably certain, the names and cultures nearly related are entered separately as parts of a homologous series of forms without claiming identity. As more fully discussed elsewhere, forms morphologically nearly related may differ so greatly in physiological activity that the indication of positive identification with a type might occasionally be seriously misleading. The further policy of allocating to their proper group in this key the specific names under which no cultures have been identified is believed to be desirable, because these specific names are already so used in the literature as to force consideration of them upon the investigator of either substrata or of biochemical processes. It is impossible to obtain type material for many of these forms. Some of them have not been reported since the original description. If, therefore, identification of the group to which they belong is possible, the investigator will have some knowledge of their significance.

Inclusion of a series of forms in a group under a single number with lettered subnumbers does not necessarily imply identity, since strains not separable on the basis of the published description may, in fact, have presented contrasts apparent to the describers and correlated with determined biochemical activities. The large number of described species which are here included in certain groups rather points to these groups as aggregations of related races or strains in which there is great

variation in activity and that this variability is expressed in differing shades of color, in vigor or luxuriance of growth, in size of the various fruiting elements, or in the extent of the changes induced in the substratum or in the tolerance of the organism for special conditions of growth. While many of the actual forms allocated to such a group may be difficult to identify, a culture exactly reproducing the conditions of an original description is occasionally encountered in a manner to justify the describer. Further, the incomplete character of any such survey as this must be fully recognized, hence the addition of further hundreds of collections will doubtless remove many of the uncertainties necessarily indicated in this Key.

MATERIAL FOR STUDY AND IDENTIFICATION

An aspergillus occurring upon an accidental or natural substratum, if belonging to one of the large and well marked groups, may commonly be identified to the group from a fresh specimen and many of them can readily be identified to the group from dried herbarium materials. Within the group, differences in composition of substrata produce marked contrasts in colony appearance, quantity of growth, colors produced, measurements, and in abundance of conidiophores and conidial masses. Drying for herbarium purposes changes the colors and renders the hyphal masses so fragile that the morphological details necessary for identification within the group are more difficult to determine. Although accurate placing of such materials is occasionally possible a much more satisfactory identification may be reached by transferring fresh material to culture media, followed by purification of the cultures so that they present single species or strains for intensive study.

Two types of material are regularly encountered by one undertaking the study of Aspergilli, the culture already isolated by another worker, and the moldy substratum with its natural mass of microorganisms often representing several or many species. In either case the nature of the Aspergilli observed must usually be sought in fresh cultures made by the one responsible for identification. In undertaking this cultural study a series of problems is encountered.

MIXED STRAINS

In the routine conduct of cultural work, contaminations of cultures of one species of Aspergillus with colonies of other species or other genera are very common. The conidia of many molds are exceedingly light

and are carried freely in the air. Entire exclusion of such contamination is difficult. In dealing with such contaminations several problems arise and different procedures are possible. A colony of a single Aspergillus well established usually inhibits the growth of other species developing in the surrounding medium as described and figured by Zeller and Schmitz (38). Even when invasion occurs the effects are so distinct as to leave no deception. When, however, the contamination with spores or mycelium is carried in the inoculum and so placed as to germinate in intimate contact with the organism desired, the colony resulting may assume the character of either organism with the other present only as a feeble mycelium, yet perpetuate both species so that the submerged species is able to reappear in some later transfer upon media favorable to it. In other cases, the dominant species may grow and fruit in an erratic manner deceptive in suggesting a reaction to the medium or other physical factor. Mixtures have been encountered in which mycelia sterile under all conditions tested have become so intimately mixed with the mycelium of an Aspergillus or Penicillium as to persist through many generations without apparent effect in the earlier stages of growth of the Aspergillus, but developing as an overgrowth of sterile mycelium in very old cultures. When such colonies are observed, great care is necessary to obtain a true picture of the physiological activity of an organism under experiment, since the contaminating organism may induce marked changes in the physiological activity of the species supposedly pure. Again, in less common cases the two may grow and fruit together without apparent inhibiting effects. The most difficult form of mixture, however, is the development of two closely related species or varieties when transferred in masses of spores making intimate mixtures. Such difficulties as these make some form of purifying cultures essential.

PURE STRAINS (FOR CULTURE METHODS SEE PAGE 39)

Cultures presenting a single strain rather than several strains or species may be obtained by several procedures. In addition to the usual dilution method of culture, one standard practice is the making of single spore transfers by some one of the methods which insure by subsequent examination that a single conidium is obtained and placed in a culture medium under conditions which make possible a complete study of its development. After single spore isolation, it is commonly assumed that all question of purity of culture is removed and that subsequent mass transfers will remain pure. The theoretical advantage of the single

spore transfer is very largely offset by the ubiquity of mold spores in the air of working rooms and in contact with everything handled there. Unless the culture produced in this way is perfectly handled, the chance of subsequent contamination is great. The worker is tempted to depend upon the purity of his original transfer rather than upon the tangible evidences of purity which are before him in every culture.

SINGLE HEAD TRANSFERS, MYCELIAL TRANSFERS

Two other practical methods of getting and maintaining pure cultures may be used readily. (1) The transfer of spores from a single head having the desired characters is accomplished by wetting the tip of a sharp needle in a sterile medium, then while working under a good handlens (for example, a Hasting's triplet 10X) touching lightly the surface of the selected head and finally stabbing the needle into a substratum already prepared for growing the organism. (2) While using the same handlens, the outermost tips of a series of vegetative hyphae, submerged in the medium at the edge of a young and growing colony beyond the area of spore formation, may be removed and planted in a fresh culture tube or petri dish already prepared with the proper nutrient medium. By proper selection the vegetative cells so taken may usually be restricted to parts from a single primary radiating hypha.

The resulting colonies in every case, whether single spore, single head or hyphal transfer, must be scrutinized as to vigor, uniformity, and consistency of growth and fruit formation, but if such care is properly taken and if numerous transfers are made and studied, either method is believed to be entirely safe as a basis for working intensively with Aspergilli.

TRANSFER NEEDLES

In handling these transfers, we have used with satisfaction for many years a no. 18 or no. 22 nichrome wire thrust into a slender brass tube so that only about 15 mm. are exposed. The point of this wire is then ground down to the sharpness and smoothness of a needle. This instrument can be heated to redness in the flame a great many times with only the occasional necessity of resharpening. It thus has many advantages beside cheapness; it is firmer and takes a better point than platinum: it stands sterilization in the flame, which promptly destroys the usefulness of steel, and it can be made any size or length to suit the workman's purposes.

CONTAMINATIONS: BACTERIA

Freedom from bacteria is essential to uniformity in the appearance and in the reactions of molds. Colonies infected with bacteria may be unchanged in character, but usually show marked physiological differences from colonies free from contamination. Associative action may have important effects upon both organisms. Sartory (39) discussed without identifying a species producing ascospores, but only when accompanied by a particular bacterial associate. Nevertheless a symbiotic colony must not be allowed to masquerade as a pure culture representative of a species. The next contaminated colony of the same species of mold but with different bacteria may present a very different picture. These conditions have been met often enough to make the emphasis upon freedom from bacteria essential in study of this group.

MITES

Mites are common associates of molds as they occur in nature, hence the worker who handles moldy substrata, in isolating his organisms must constantly watch for the invasion of his cultures. Mites are in size commonly just about at the limit of visibility by the unaided eye. One accustomed to them will detect them readily, but until once seen and the appearance of their depredations understood, they have passed unnoticed for considerable periods by persons who are otherwise good culture workers. Mites will crawl from petri dish to petri dish, leaving behind them a trail of bacterial and mold contaminations as well as streams of eggs which develop rapidly into mites that actually destroy the colonies. Since mites have preferences as to food, some species are invaded and others avoided. To reach an attractive food supply a mite will frequently go through a cotton plug as ordinarily made and occasionally seems to get through even a paraffined plug. As a factor in the mixing of strains of molds in a laboratory collection, mites must not be ignored.

Similar mixing and contamination occurs frequently whenever a laboratory becomes infected with ants, roaches or other insects.

MOLD DISEASE OF A. NIGER

A mold disease of *Aspergillus niger* is common in cultural study. The colonies of the Aspergillus are overrun with an olive-green Penicillium belonging in a section of the *P. luteum-purpurogenum* (Biverticillium Biourge) group close to *P. rugulosum* Thom. This mold invades

the mycelial felt, winds its hyphae about the stalks, and fruits in a radiating series of short conidiophores about the heads and upper halves of the stalks. The black fruiting surface is commonly completely covered with the olive-green conidial masses of the Penicillium. Where *A. niger* is grown in tanks for acid formation, spots infected in this way are killed and disintegrated, thus interfering with fermentation, while the infected areas may be seen to drop out when the blanket or felt of *A. niger* is lifted from the surface of the liquid. Other species when inoculated have been irregularly affected, some strains of the same species were attacked and others apparently immune.

REPLACEMENT BY CONTAMINATING SPECIES

Other species of fungi invade mold cultures and some of them become so intimately associated with the mycelium and conidia of particular species that it is difficult to eliminate them by the ordinary method of transferring spores with a loop or wire to streaks or stabs. Proper dilution culture presents the possibility of elimination but demands careful examination and selection from the resulting colonies. The progressive replacement of a particular species, by invading organisms, is constantly encountered in examining cultures passed from laboratory to laboratory. The original form may disappear without detection if the displacing species has a superficial resemblance to it or if no detailed examination of successive transfers is made by a worker really familiar with the specific characters. The physiological or biochemical investigator obtaining such a culture assumed as correctly named will be seriously misled in the interpretation of his experimental results. No culture should be used for such an investigation without being fully identified at the outset and having its proper appearance and reactions sufficiently studied to insure the reliability of the results during the progress of the work. In other words, before undertaking to do biochemical or physiological work, sound scholarship calls for adequate precautions in the identification of the original material supplemented by such mastery of its morphology and variability as will insure its maintenance in proper condition.

CHAPTER IV

Culture of Aspergilli

CULTURE MEDIA

Pure culture upon known substrata is, therefore, almost essential to the identification of Aspergilli. Since the morphological response to diverse nutrients and especially to the stimulus of mixtures of other molds and bacteria growing with any particular species has already been shown to be great, the study of each strain or species in pure culture in media of known composition is practically necessary. As a routine medium for this purpose the following formula (40) originally adapted from Czapek by Dox (41) has been used with occasional minor variations in quantities which apparently did not affect the reactions (see succeeding paragraphs). Cultural information given in this paper is obtained from growth upon media produced by this formula unless otherwise specified.

Czapek's solution agar

As originally proposed		*Modification not affecting Aspergilli*
Water	1,000 cc.	1,000 cc.
Sodium nitrate	3.0 grams	2.0 grams
Potassium phosphate (K_2HPO_4)	1.0 gram	1.0 gram
Magnesium sulphate	0.5 gram	0.5 gram
Potassium chlorid	0.5 gram	0.5 gram
Ferrous sulphate	0.01 gram	0.01 or trace
Sucrose	30.0 grams	30.0 grams
Agar agar	15.0 grams	By test of sample 12.0 to 20.0 grams

In making this culture-medium the neutral potassium phosphate is preferred to the acid form of the salt, since with the neutral salt the final reaction of the medium is neutral or only very slightly acid. Czapek's solution agar is not offered as an optimum substratum for any particular species, but as a mixture approximately neutral in reaction, which is readily made in any laboratory in fairly uniform manner, and which permits moderately vigorous growth of nearly all saprophytic Aspergilli. The quantities of mycelium and conidia produced by many forms in other media are much greater, but for comparative study, a

moderate growth of the majority of the species is more useful than the great masses of mycelium and conidia which are readily obtained by using enriched substrata.

SUPPLEMENTARY MEDIA

Culture upon Czapek's solution agar has been supplemented by culture upon wort agar, potato agar, bean agar, potato and carrot plugs, milk, wood, licorice root, soil, starch media, bacteriological meat extract agar, various types of gelatin media, and media with increased salt, sugar, and acid content.

SYNTHETIC MEDIA

The substitution of so-called synthetic solutions for "natural" substrata, such as potato, carrot, meat, and their extracts and infusions dates back to Raulin (42), who sought to replace the ash of yeast used by Pasteur, by providing the salts essential to growth in the following formula for "Raulin's solution."

Raulin's solution

	grams
Water	1,500.0
Cane sugar	70.0
Tartaric acid	4.0
Ammonium tartrate	4.0
Ammonium phosphate	0.6
Potassium carbonate	0.6
Magnesium carbonate	0.4
Ammonium sulphate	0.25
Zinc sulphate	0.07
Iron sulphate	0.07
Potassium silicate	0.07

However useful this solution may have been, the complexity of the mixture, with the repetition of several elements in it, led others to seek in much simpler formulae to present each element but once. Raulin's solution was an advance in representing a medium of approximately known composition, hence theoretically capable of reproduction anywhere. In doing this, he established a sound ideal of culture. Accepting the idea of simplification, however, succeeding workers adjusted the combinations and concentrations of the various nutrients used to the particular mold or molds in culture, or to the fermentation process under investigation. Many formulae are thus available; most of them include the same series of chemical elements, combined, however, as different

salts, or salts in varying concentration. These variations may be typified by some of the revised forms of Czapek's formula. Dox (41) and Currie (see Thom and Currie, Jour. Agr. Res. 7: 2. 1916) prefer to make up this solution with monopotassium phosphate KH_2PO_4 instead of K_2HPO_4 as used in our work. In the course of much experimentation, Currie concluded that equally good growth of *A. niger* and related forms could be produced with the potassium chlorid and magnesium sulphate cut in half (to 0.25 gram per liter). Dox reduced the sodium nitrate to 2 grams; a practice used for considerable periods by us without affecting the colony appearance or mold structure appreciably. Later, Currie in seeking special results in connection with the citric acid fermentation proposed to use in each 1,000 grams of solution, 125 to 150 grams of sucrose, 2 to 2.5 grams of ammonium nitrate, 0.75 to 1 gram monopotassium phosphate, and 0.25 gram of magnesium sulphate to which he added sufficient hydrochloric acid to produce a hydrogen-ion concentration of pH 3.4 to 3.5. This formula was not proposed to maintain normality, or stock cultures, since he recognizes the necessity of iron in a normal medium, but it is characteristic as a formula produced for a very special purpose. Molliard (43), however, studying the same problem varies the formula as follows: To the liter of solution, sucrose 46.6 grams, ammonium nitrate 0.356 gram, monopotassium phosphate 0.08 gram, magnesium sulphate 0.08 gram, iron sulphate and zinc sulphate each 0.0046. Obviously the results of various workers are influenced greatly by the details of handling practiced in their laboratories. In contrast, to these dilute formulae Duggar (44) gives under the name of Richards' solution the following:

Richards' solution

	grams
Potassium nitrate	10.0
Monopotassium phosphate	5.0
Magnesium sulphate	2.5
Sucrose	50.0
Water (distilled)	1,000.0

Again, Steinberg (45) selected as his basal medium under the name Pfeffer's (46) solution: Water 1,000 grams, cane sugar (commercial "Crystal Domino") 50 grams, ammonium nitrate 10 grams, monopotassium phosphate 5 grams, magnesium sulphate 2.5 grams, ferrous sulphate trace. This is also much more concentrated than the Czapek formula used by us. In using these synthetic formulae, agar may be added to make a solid substratum, or substitutions may change the form of carbohydrate, or nitrogen offered for nutrition.

WORT

In general culture work involving unknown organisms to be isolated from natural substrata, few media have ever proved more useful than the so-called "wort." In places with the brewery accessible, the wort, or beer wort could be obtained readily, concentrated to any desired density and used either as a liquid or with agar as a solid substratum. Since the basal material, wort, was less readily obtained in many of our laboratories, we inaugurated experiments toward the production of a substitute formula using as a base the Difco dried malt extract, long used by Blakeslee for Mucors, and checking results step by step by the use of brewery wort. This was published by Reddish (47): Dissolve 100 grams dry extract of malt in 900 cc. distilled water; make to 8° Kaiser (saccharometer) by adding distilled water (approximately 100 cc.). Adjust reaction to +1.5. Cook in autoclave fifteen minutes at 15 pounds pressure. Filter through folded filter paper, tube and sterilize at 15 pounds for ten minutes, or ten pounds for 15 minutes. It should not be sterilized at the same or higher temperature and pressure as that at which it was cooked, for a precipitate will again come down. Agar can be made by adding 1.5 per cent agar to the solution of malt extract after it has been adjusted to +1.5. Heat at 15 pounds pressure for twenty minutes, then filter through cotton until clear. The same precautions as to sterilization must be observed.

FORMS OF CULTURE

Cultures for grouping and identification of Aspergilli should take two forms, petri dish cultures and slants. The petri dish (so-called plate culture) may be used for dilution culture, for stab or for streak. In case dilution is desirable, petri dish cultures of Aspergilli are only useful when a very small number of colonies develop on each plate. Where numerous spores begin to form colonies at the same time in a petri dish the identity of the individual colony is commonly lost and details of development can not be followed. In questionable mixtures large numbers of plates are required to reach proper dilutions for study. If bacterial contamination is probable, acidification of a portion of the plates is desirable. In such a series of plates the examiner has a choice of different dilutions, different aggregations of colonies from which transfers may form the basis for pure culture. In case the material is in such condition that transfers of heads or mycelium after handlens examination are possible, the petri dish poured and cooled, then used for stab-

bing or streaking purposes in the same way as a slanted tube of agar or gelatine is most satisfactory.

In the petri dish, the development of the colony and of the individual heads can be followed with the compound microscope, making possible a much more accurate conception of the morphological relations of vegetative hyphae and fruiting apparatus than can ordinarily be obtained from mounted slides. In many species, heads upon isolated colonies are only developed so far from the edge of the colony that observation is difficult on account of the mass of mycelium produced. In dealing with these forms, a petri dish containing several colonies becomes very useful, since the mycelia of two colonies as they approach each other commonly stop growing to leave a narrow sterile band of medium between the colonies, thus permitting a scattering group of stalks to develop to the very edge of each colony and extend out over the sterile space where lighting effects are favorable for focussing and observation. In examining cultures in this way the probability of contaminating them must be assumed, hence a transfer should be made from every colony deemed worthy of study before the plate is exposed, unless an ample reserve of duplicate cultures is known to be available.

SLANTS

The slanted tube of agar, gelatine or other substance, closed by a well rolled cotton plug may be handled freely with little danger of contamination. The mold colony developing in such a tube may be followed with a handlens and gross features of its development recorded, such as color production, liquefaction, crystal formation, and development of sclerotia or perithecia. Several such cultures should be made to preserve any mold desired for study. In this way, comparison of a series of tubes gives a safer basis for a record than single cultures, while some tubes may be held unopened as reserve cultures. A slant properly handled may sometimes be kept for years in pure and viable condition as a source of transfers, but it is better policy to add one or more new stock tubes whenever the reserve stock of unopened tubes runs low.

HANGING DROP

The cluster of conidia transferred to a drop of medium suspended in a cell has been much used by some groups of mycologists. In our experience, it has one real value; it gives opportunity to study the germination of a spore and the formation of the first few branches of mycelium. In such groups as Aspergillus and Penicillium, the development

of fruiting hyphae in the hanging drop lacks the characters found in the usual type of colony, hence is unsatisfactory for study beyond the early stages of development.

STORAGE OF CULTURES

To avoid the loss of valuable stock cultures, it has been found desirable to store part of them at room temperature in one or more separate locations, part of them in the ordinary ice refrigerator and a third set in cold storage at about the freezing point. Where storage slightly above the freezing point is available, all of the Aspergilli in our collection have been safely held by transferring once each year. Experiments in the storage of molds of other genera do not justify general use of such storage temperature for so long a period. In Penicillium, for example, the ice refrigerator at 11° to 16°C. has been more satisfactory.

Cultures on Czapek's solution agar store well under these conditions. Bean and potato agar slants have been equally satisfactory. Experiments with Bainier's method of culturing molds upon licorice root, upon carrot plugs as used by Mangin, or upon stems and pods of bean, pea, of alfalfa show that a wide variety of "natural" substrata are decidedly favorable for use in keeping stock cultures viable.

LONGEVITY OF ASPERGILLUS SPORES

Records of the longevity of specimens of Aspergillus in storage have occasionally been published. McCrea (48) reported conidial material of *A. oryzae* viable in a tube stored twenty-two years. An *A. flavus* tube obtained in Wehmer's laboratory remained viable after nine years, but appears to be dead at twenty years. Incidental examination of great numbers of stored cultures shows that Aspergilli commonly survive holding periods of several years, but that such survival is dependent upon maintenance of favorable conditions, instead of being a specific quality uniform to the species. Thus far, conditions leading to such longevity have not been defined closely enough to be adopted if one seeks to insure survival in a series of cultures.

EXAMINING ASPERGILLI IN CULTURE

Microscopic equipment recommended

A good microscope with an oil immersion objective is almost essential to the study of Aspergilli. A few of the observations can be made with the naked eye, some with the handlens, others with low magnifications,

but careful use of a good high power, preferably an oil or water immersion, insures a safer series of observations for the final details. A well calibrated micrometer for measuring structures is likewise essential.

To be made directly upon the colony growing in petri dishes

Using a handlens. Morphology of colony; presence or absence of aerial vegetative felts; relative depth and density of colony growth at center and at periphery of older colonies; color of young mycelium, of the underside or reverse of the colony, of media, of young and mature stalks and heads; relative size or uniformity of heads.

Using low magnifying objectives of the compound microscope. Measurment of heads in the growing colony, relative size and uniformity of masses of conidia, variations of shape and size of heads, and in length and course of stalks, with their markings and their relation to substratum.

To be made from microscopic mounts

By using a sharp needle under a good handlens, groups of conidiophores may be separated, transferred to slides, treated with a drop of alcohol to remove air bubbles, mounted in water, dilute glycerin or other mounting fluid with or without stain, and covered for examination. If carefully separated and mounted, such a preparation can be examined first with a series of dry objectives, followed by the oil-immersion. We have preferred to use the Zeiss 3 mm. apert. 1.30 apochromatic with a 12X compens. ocular for final determinations.

1. The presence of a differentiated foot-cell may be regarded as the diagnostic character separating border-line species from Penicillia, otherwise closely resembling Aspergilli. The entire conidial apparatus (foot-cell, stalk, and head) with its appendages is to be regarded as an asexual fruiting organ.

2. The color, thickness and markings of the stalk are to be examined with a good oil-immersion objective or its equivalent; pitting or apparent roughening when viewed with low power lenses is characteristic of certain groups and absent in others; colored layers, especially outer layers are characteristic of certain groups; the broken ends of stalks crushed while mounting, and the general proportions (length, diameter, nature of thickenings) all give useful comparative information.

3. The vesicle, its shape, measurements of length and diameter, relation to stalk, location and extent of fertile area, color, and especially the thickness, coloration and perforation of walls.

4. Sterigmata: Whether in one or in two series, or both conditions in the same or adjacent heads, the relative size, shape, and color of each and their relation to each other and to the vesicle. Where two series occur the measurements of the primary sterigmata are usually much more significant than those of the secondary group, although both should be recorded.

5. Conidia: The conidia are cut off, they do not bud out, hence they are theoretically cylindrical at first, however quickly they may become elliptical or globose. Care in interpreting surface appearances of conidia is essential; for example, with low magnification the walls of conidia in most strains of *A. flavus* and *A. oryzae* appear to be echinulate, with high magnification and careful focussing such a surface usually proves to be pitted and grooved instead of bearing spinules or tubercles upon a smooth basal wall as the color bars and tubercles of *A. niger* or *A. tamarii* This determination separates two large groups of forms and this separation is confirmed by biochemical determinations by various investigators.

CHAPTER V

Physiological and Biochemical Studies

The various species of Aspergillus have been favorite organisms for laboratory investigation of physiological and biochemical problems. No attempt is made here to cite in full the enormous literature resulting. Certain representative papers will be discussed with such references as may assist in locating the sources of information with the discussions in full. These researches range from metabolism studies in which pure cultures of the mold have been maintained and tested for their power of growth and reproduction against media of supposedly known composition to the measurement of the capacity of a particular organism to produce quantitative changes in a particular product.

Such formal investigations of Aspergilli may be said to begin with the studies of the tannin fermenting activities of *A. niger* by Raulin, Van Tieghem and their co-workers, in the years from 1860 to 1870. Van Tieghem during this period extended his observations to include other species, but all of these reports are characteristic of most of the early work and unfortunately of much recent work in failure to describe the organisms closely enough to insure recognition of the species used when other investigators attempt to repeat the work. Before Van Tieghem, most of the observations upon the activities of Aspergilli consist of notes and observations of species found under natural conditions in which the cultures were assumed to be pure and to be specifically responsible for apparent effects upon the substrata.

Although all of these studies are fundamentally investigations bearing upon the metabolic activities of these molds, in a general way two lines of development may be separated. In one, the activities of the organism are tested against nutrient media as the basis for growth, by comparative study of the colonies produced. Basal nutrient media are established and the materials to be tested are added under conditions which make possible a quantitative study of their availability for fungous metabolism. The physiological responses of the organism to stimulations of this kind have contributed greatly to our knowledge of protoplasm. In the other series, what may be called the external activities of Aspergilli have been studied. Aspergilli grown in rich and favorable nutrient substrata are found to produce changes in such substrata, presumably,

primarily for the purpose of rendering substances available for utilization by the mold but greatly exceeding in quantity the possibilities of absorption by the growing colony. Extensive fermentations are thus produced, some of which have proved industrially useful. In the first series the physiological responses of the organism are paramount and for convenience these will be referred to as nutrition studies; in the second the quantitative ability of an Aspergillus to cause fermentation or decomposition is measured and applied to problems of prevention or of utilization; this will be discussed as enzymic and fermentative studies.

NUTRITION OF ASPERGILLI

Studies of the nutrition of the Aspergilli are numerous. The range of nutrients utilizable by different species varies, but the cosmopolitan saprophytes, such as *A. niger*, have been designated omnivorous (Hochapfel (49), p. 221) with considerable reason. Survey of the distribution of Aspergilli in nature, however, clearly indicates the special adaptability of particular species to thrive in environments presenting more or less definable conditions of growth. Experimental work to establish or define the limits of tolerance for the various species of the group became abundant with the development of the cultural line of attack upon microbiological problems largely under the influence of Pasteur.

This long series of investigations begins with Raulin's proposal of a so-called "synthetic" medium (see page 40), a mixture into which he undertook to introduce in soluble form all of the elements demonstrated at that time by analysis as present in the ash of yeast and mold mycelium. The elements agreed upon as essential were hydrogen, oxygen, carbon, nitrogen, phosphorus, sulphur, potassium and magnesium, with debate as to iron, calcium, zinc and silicon. As to iron, the weight of evidence favors its inclusion; the other three elements are frequently omitted from formulae as published. It was early recognized that other elements were absorbed when present, but their utilization by the molds was more difficult to demonstrate.

Fungi as in the case of green plants may be assumed to exercise a selective absorption from the nutrient substratum resulting in marked concentration of certain elements, but at the same time they are found to take up elements not commonly listed among those essential. In green plants (Palladin (50), McHargue (51)) the list of elements present in plant tissues has grown to considerable proportions. Palladin names 31 in all. McHargue in his recent paper has determined as parts per

million the quantities of iron, copper, zinc, manganese, cobalt and nickel in a series of products, both animal and vegetable. These workers indicate clearly that the quantities present are physiologically appreciable and that we have little evidence to affirm or deny a function to some of these elements. With reference to the fungi, very much remains to be done.

OXYGEN RELATIONS

Aspergilli are in general rather strictly aerobic. A colony inoculated into a liquid or semi-liquid substratum will grow as a blanket of mycelium upon the surface or bending and buckling will form protuberances into the fluid somewhat, but does not ordinarily grow freely when immersed. Fruiting occurs on the surface only, in fluid media or in semi-liquid substances because the conditions presented, exclude free access of air to the interior of the mass.

Conidia of some species inoculated into liquid which is disturbed, thus preventing the formation of a surface felt or blanket of mycelium, grow to produce long, loose, colorless streamers of submerged hyphae, but without identifying characters. When such mycelium has been removed to solid substrata and incubated, it has commonly proved to be *A. niger*. Few other species of Aspergilli have been obtained from such situations by us.

When a substratum is non-liquid but porous some species will penetrate deeply into the mass. In bread, for example, *A. repens* and, perhaps, other members of the *A. glaucus* group will frequently penetrate the entire loaf, producing both perithecia and conidia in the air cells. Such molds as *A. niger* and *A. flavus* usually remain as a surface blanket of mycelium bridging over the narrower cracks or openings rather than entering and fruiting deeply in the mass.

In soil cultures in the laboratory presenting a fairly close textured mass, but permitting access of oxygen to the spaces among the soil particles (Thom and Church, loc. cit., 1918), *A. nidulans* was found fruiting 5 cm. below the surface of sandy soil and *A. terreus* 3 cm. below the surface, although neither of them fruited below the surface in a clay soil which packed more closely. *A. flavus*, *A. fumigatus*, *A. tamarii*, *A. nidulans*, and *A. terreus* were recovered in culture from points 5 cm. below the surface of the clay soil showing that mycelium had penetrated the mass to depth at which fruiting was no longer possible. Mycelium appears to grow fairly freely at oxygen tension below that required for conidium formation.

WATER IN THE NUTRIENT SOLUTION

In pure cultures, most of the Aspergilli grow readily throughout the range of concentration commonly used in laboratory media; this runs from extreme dilution, such as Currie's modification of Czapek's solution (page 41) to solutions fairly rich in salts and carbohydrates. A survey of the occurrence of these species in nature brings out some striking contrasts. *A. glaucus* of Link was the green Aspergillus of the phanerogamic herbarium upon flowering plants imperfectly dried. In the study of moldy foods, feeding stuffs and miscellaneous materials, the various members of the *A. glaucus* group have been shown to become the first molds active in cornmeal (Thom and Le Fevre (52)) as the water content rises above 12.5 to 13 per cent. Members of the same series are the dominant molds on salted meats where salting or brining has produced very high concentrations of sodium chloride; they are equally dominant on the surface of jams and jellies where high concentrations and osmotic pressures are produced by sugars. Samples of corn stored at moisture content only slightly above the critical percentage have been examined and every kernel found infected and showing green heads within the germinal area; in the molding of hay and corn-fodder, as well as the other products mentioned, the members of the *A. glaucus* group are the first molds to become active as the moisture percentage rises above the critical point at which molding entirely ceases.

In studying *A. herbariorum* (?) Fraser and Chambers (27) found that concentrations of sugar up to 40 per cent were favorable for species of this group to grow and fruit quickly. In our work, members of this group are found usually to dominate even in grossly mixed cultures when 40 to 50 per cent of sugar is introduced. In cases where the presence of Aspergilli of this group have been suspected, it has been repeatedly possible to permit them to develop by adding such an excess of sugar to the substratum, as either hampered or suppressed competing forms, whereas in dilute media the quicker and more abundant growth of other molds completely submerged the slower growing colonies of *A. repens*. Members of the *A. glaucus* group are then the dominant molds in carbohydrate containing products under natural conditions of extreme physiological drought, whether that condition results from mere scarcity of water, or from high percentages of such osmotically active agents as salt and sugar, as in brines or syrups.

In cornmeal a rise of 3 to 5 per cent above the critical moisture content, permits *A. flavus* first to appear at about 16 per cent, followed quickly by *A. niger* and numerous molds at 19 to 20 per cent. Growth

at these low moisture percentages, even though it is readily observable, is slow in comparison with growth at more favorable moisture content. In the fermentation of soy beans roughly 50 per cent moisture is found to give quick and satisfactory development of the *A. flavus* type of organism. Bread at 35 to 38 per cent moisture, molds rapidly only if surface evaporation is cut down to produce a very humid atmosphere.

If the moisture content rises above 50 per cent, Aspergilli grow readily in pure cultures. Under natural conditions, however, bacteria and other molds which grow more rapidly commonly obscure their development unless the substratum presents conditions selectively favorable to particular species, such as high sugar, tannin or acid content. Failure to find a species in a particular substratum under specific natural conditions does not prove its inability to grow there, but may indicate merely its failure to compete successfully with other forms also present. Conversely dominance of a particular species in a specific situation does not necessarily imply optimum growth conditions for it, but may merely indicate greater tolerance for those conditions than that possessed by competing forms.

In studying Aspergilli as a cause of decomposition or fermentation under natural conditions, the percentage of water present, calculated against the total weight of the mass, is less valuable than the percentage and character of the osmotic substances in solution in the moisture as distributed through the substratum.

REACTION OF NUTRIENT MEDIA

Although the cultures reported here have mostly been made in media originally nearly neutral (pH 7.0), most workers prefer to use acid media in the isolation and study of particular molds. Two reasons are given: (1) Acid in the medium tends to eliminate or reduce the development of such bacterial contaminations as may be present in the inoculum and (2) most of the molds grow better in acid than neutral or alkaline media. Webb (63) goes further, however, and finds that the conidia of the molds he used, including *A. niger* (with the exception of one species of Fusarium) showed increasing percentages of germination as the acidity rises from pH 7.0 toward a maximum which is about pH 3.0 for *A. niger*. From this point such percentage of germination falls rapidly with further increase of hydrogen ions. The range of growth found for *A. niger* was pH 2.8 to 8.8, thus showing much less tolerance for alkaline than for acid growing conditions.

In dealing with Aspergilli, little difficulty is encountered in securing

the germination of transfers in neutral media after cultures are once isolated. Acid media are favorable for obtaining the initial culture from natural substrata and, perhaps, some advantage in preventing the growth of accidental bacterial contamination. Once in culture, colonies beginning to grow in media containing a fermentable carbohydrate quickly produce acidity, which in no case seems to rise above a maximum without interference with growth. Aside, therefore, from facilitating obtaining the initial culture and possibly hastening the germination of the transfer only slightly, there appears little argument for the use of acid media in the miscellaneous culture of Aspergilli.

NITROGENOUS NUTRIENTS

The Aspergilli have been called omnivorous as can be essentially verified by the wide range of culture substrata upon which they grow freely. In the preparation of nutrient mixtures, such as Czapek's, Raulin's, Pfeffer's, or Richards' solution, the element nitrogen may be supplied in several forms. In Czapek's solution we have used sodium nitrate. As a result of experiments indicating the production of a greater mass of mycelium from its use, others have preferred to substitute an ammonium compound in the belief that nitrogen is more readily available as ammonia than as nitrate. Molliard (54), however, in studying alcoholic fermentation by *S. nigra* reports greater activity from the addition of potassium nitrate than from the use of ammonium chloride in the same quantity. In dealing with strictly saprophytic molds, we have found few, if any, species which fail to grow readily when presented nitrogen as found in sodium nitrate in Czapek's solution, but as already indicated greater masses of mycelium are produced with many other substrata. Kostytschew and Tswetkowa (55), have recently discussed the mechanism of utilization of nitrate (KNO_3) in such a medium, finding reduction to nitrite to occur regularly. Zaleski and Pjukow (56) tested the availability of nitrogen in various forms using as a basal solution 6 to 7 per cent glucose, 0.05 per cent magnesium sulphate, 0.2 per cent potassium phosphate to which nitrogen was added variously as ammonium sulphate, Witte's peptone, and individual amino-acids, with incubation at 33° to 34°C. The results were tabulated on the basis of the weight of mycelium produced in a given growing period. In general, the ammonium compound was found more available than the amino-acids.

The divergence of views presented in various investigations is largely dependent upon the ends sought by the investigator. The different

criteria may be the volume or weight of the mold colony, the quantity of a particular enzyme, the extent of change induced in a substratum, or the worker's judgment as to the appearance or normality of the colony itself. Obviously no final judgment as to sources of nitrogen is possible, since the Aspergilli in nature utilize the widest range of plant and animal remains for growth and multiplication. They secrete enzymes capable of digesting nitrogenous products of great variety, in addition to their ability already demonstrated to absorb many kinds of nitrogenous substance when encountered in diffusible form.

ASSIMILATION OF ATMOSPHERIC NITROGEN

There is a wide diversity of views as to the possible assimilation of atmospheric nitrogen by fungi with evidence that a few species really fix small amounts.

Latham (57) using *A. niger* in a series of cultural studies upon media of known composition found increases in the total nitrogen present which she attributed to the fixation of free nitrogen by this species. This fixation was lessened and finally inhibited by the addition of increasing quantities of zinc sulphate.

FUNGOUS PROTEIN ACCUMULATED

Experiments with hydrolyzed straw were made by Pringsheim and Lichtenstein (58) for the purpose of adding to the protein content of this material as a feeding stuff. The straw was inoculated with a non-pathogenic strain of *A. fumigatus*, which was allowed to develop abundant mycelium, after which the material was dried and used for feeding sheep, cattle and rabbits. The total protein content was increased in certain of these experiments from less than 1 to 8 per cent. About 40 per cent of the protein of the moldy straw was utilized by sheep under experiment. Although it was shown to be possible as a war-measure to produce protein for feeding purposes in this manner and make it palatable by proper combinations, the process does not appear to have been developed further. Such experiments as this clearly show the ability of molds to change the percentage relations of protein, fat, sugar and starch in foods and feeding stuffs.

The essential elements, phosphorus, sulphur, and potassium, have been the subject of several investigations utilizing *A. niger* as a test organism.

PHOSPHORUS

Dox (59) found his cultures able to utilize phosphorus from a wide variety of phosphorus-containing substances, but that this element was not available in trivalent form.

SULPHUR

Armstrong (60) found *Aspergillus niger* able to utilize a wide range of sulphur compounds.

POTASSIUM

Waterman (61) and Molliard (62) studied the availability of forms of potassium; Waterman also studied the replacement values of several elements and combinations against potassium comparing especially rubidium and manganese.

Molliard (62) in a recent paper reports experiments upon the influence of sulphur and potassium upon the structure of the mold colony. By reducing the sulphur and potassium in the medium separately to $\frac{1}{100}$ of the usual amount, or adding sufficient acid in the medium, the mycelium produced remained sterile. If sulphur was reduced, it became a thick blanket-like mass taking up much of the liquid in the meshes between fine hyphae; if potassium was reduced, the cells of the sterile mycelium became coarse and vesiculose. The conditions observed disappeared when the proper nutrients were added to the experimental lots. The possible agency of acidity in producing these effects was excluded by proper checks.

METALS IN CULTURE MEDIA

In the series of metabolism studies which have followed the work of Raulin and Van Tieghem, and which commonly have used *A. niger* as the principal species for study, much attention has been given to the effect of the metals, iron, and zinc upon the organism. The experiments of various investigators have yielded more or less conflicting results, but in general indicate a stimulation of growth by very small quantities of zinc and that iron is commonly essential to normal fruiting, and certainly so in *A. niger* (Currie, personal communication). Javillier (63) for example, reports that the addition of one part of zinc to two million of medium increases the volume of growth of *A. niger* fifty-eight times. Since the quantities of these elements necessary are very small, expressed in parts per million, it may be readily shown that the precautions taken

by most of the workers were entirely inadequate to exclude such infinitesimal amounts. It has been often demonstrated that the quantities of many elements as found in analyses of plant ash are not uncommonly present as impurities in commercial chemicals, in such substances as gelatine, and agar or plant or animal extracts or digests. The ability of molds to etch or erode glassware is equally familiar. These minute quantities were ignored, however, in the majority of studies by the earlier workers, although protests (64) began to appear from time to time and the whole subject was reworked by Steinberg (65), who concludes that both iron and zinc are required for vigorous development of this species. He concludes (1920), however, that such other chemical stimulants as uranium and cobalt may partially replace zinc, iron, or both metals.

The possible effect of very minute quantities of metals was also investigated by Bornand (66), who reported cultures retarded by growth in vessels of silver, but not by growth in vessels of platinum or aluminium.

In general, one is more or less disappointed in seeking tangible conclusions as to the function of the less abundant elements in the nutrition of the Aspergilli. Until, therefore, this whole field has been reworked in the light of the minuteness of the minimal quantities necessary as demonstrated in the case of the so-called "vitamins" we shall not know the number of elements or combinations which must be added to the list of substances essential.

LIGHT RELATIONS

In the laboratory, no great difference is commonly noted between cultures of this group grown in closed dark incubators and cultures grown with more or less exposure to diffused daylight.

TEMPERATURE

In the cultural description of their species, recent writers have commonly determined the effect of varying temperatures upon the growth and fruiting of their Aspergilli. Tabulation of such findings would be misleading because differences in media and manipulations affect the findings, especially as to optimum temperatures. In our own cultures, strains of *A. fumigatus*, *A. nidulans*, *A. terreus*, *A. flavus*, *A. ochraceus*, *A. niger* grew well at 37°C. or higher; certain of the *A. glaucus* group preferred to grow at blood heat. Within groups as indicated by Bainier

and Sartory for their series in the *A. glaucus* group and by various workers for the *A. fumigatus* group, strains are found to vary considerably in their responses to temperature. In our laboratory cultures all species tested have grown freely between 20° and 33°C., with the possible exception of a single strain which seemed to prefer a lower temperature. As the temperature rises toward 37°C. or higher, such heat loving forms as *A. fumigatus* grow exceedingly well, sometimes overrunning and smothering all competing species. The less thermophilic forms gradually slow down as the temperature approaches blood heat until growth ceases at temperatures considerably below the death point of the spores. *A. fumigatus* has appeared in experimental work in the heating of organic masses as the mold most tolerant of higher temperatures. It has appeared to be active in heating masses at temperatures above 50°C. Takamine (67) gives the optimum temperature for *A. oryzae* as 30° to 35°C., but finds it growing well up to 40° to 42°C., although apparently not to so high a temperature as *A. fumigatus*. Nishiwaki (68) narrows this optimum figure to 33.5 to 34.5, gives a minimum as 7° to 9°C., and a maximum of 45° to 47°C.

As the temperature falls below the optimum for any species, its activities gradually slow down to become negligible at the freezing point. A few species, however, adapt themselves to the cooler temperatures of growth. A culture (4724.45) received as *A. medius* Meissner was found to grow more abundantly in an ice refrigerator at 10° to 14°C. than at higher temperatures. Other members of this same series (*A. glaucus* group) are common upon meat in moist chill rooms where they form long shimmering streamers of mycelium. In this group a few thermophilic strains have been reported, but the majority of the forms studied have grown best below 37°C.

FREEZING TEMPERATURES

A series of cultures were stored for four and one-half years in a room with the temperature irregularly maintained at about −12°C. (10°F.). At the end of the period, the Czapek agar slants were removed and cultured. Except for *A. fumigatus*, some of the slants of each species were dead. Living cultures were found in lots representing the groups *A. niger*, *A. flavus*, *A. sydowi*, *A. tamarii*, *A. candidus*, *A. terreus*, *A. versicolor* and *A. glaucus*. All of the cultures survived in the case of *A. fumigatus* only. All were dead in *A. ochraceus*, *A. clavatus* and *A. nidulans*.

In another experiment, a series of Aspergilli, especially sclerotium formers, were grown to maturity in tubes in the laboratory, packed in metal cases and exposed outdoors to the alternation of freezing and moderate temperatures of an entire winter in Washington, D. C., without apparent effect upon the viability of any culture or upon the subsequent development of the sclerotia in the forms selected.

PARASITISM: SAPROPHYTISM

Aspergilli are abundant on dead or dying plant tissue. There is little to indicate actual parasitism, however, in the examination of living and growing material. In ripe fruit, such as figs, dates, berries, nuts, or apples, or in stored bulbs as onions, leeks, lilies, etc., the line between the parasitic and saprophytic mode of life becomes scarcely distinguishable. This is equally true of the bark of trees, of secretions and exudates of many kinds in which saprophytes are abundant. Aspergilli are widely present in many plant products as they verge toward the dead or dying condition. As invading mycelia, destroying plant tissue or causing disease, few cases are demonstrable. In spite of many reports of Aspergilli in the host indexes, no attempt is made here to cite other than certain conspicuous occurrences of Aspergilli in such situations.

The presence of Aspergilli in pathological conditions observed in man and other animals is discussed in Chapter VII.

FUNGOUS STARCH

The production of starch-like products by fungi has been reported by a number of investigators. This literature has recently been summarized by Schmidt (69) with further experimental work. The so-called fungous starch is deposited largely in the thickening of the cell-walls of the mycelium and stalks and in the sclerotia when present. It is also to some extent found dissolved in the medium. In some cases it is manifestly a reserve foodstuff, but its utility to the mold has not been determined in other cases. The nature of the various products synthesized by molds and their importance in the physiology of the organisms themselves or as by-products utilizable by man present whole series of problems which have been barely touched.

CHAPTER VI

Enzymic and Fermentative Activities of Aspergilli and Their Industrial Significance

The external activities of Aspergilli referred to above are the changes in substrata due to their presence, but extending beyond the zone of activity of the organisms themselves, hence measurable by the alterations observable in the physical condition of the substratum, or the chemical changes in the composition of the substratum itself. Except as the addition of the tissue of the mold itself may be occasionally significant, these changes as far as analyzed are apparently enzymic. Some of these changes have been intensively studied, a few of them have proved industrially important as sources of by-products of value, others are commercially important as causes of decay and loss in products more valuable in the sound condition.

INDUSTRIAL USE OF ASPERGILLI

Industrial use of the biochemical activities of the Aspergilli has been restricted to a small number of species. A mold fermentation to be successful commercially must either produce a product of such price as to warrant expert service with the maintenance of pure cultures or must be based upon an organism capable of vigorous growth under a wide range of conditions. Such an organism must grow rapidly enough to overgrow and swamp such competing species as may be able to survive the conditions of culture or must be tolerant of such concentrations of a suitable inhibiting agent as will automatically restrict the flora of the fermenting mass. At best, mold fermentations are difficult to control, since more than one species will usually thrive under any particular conditions.

Among the Aspergilli such colonies ordinarily develop only on the surface of substrata rather than diffuse themselves more or less evenly throughout a liquid mass as do the bacteria, yeasts and the yeastlike Mucors of the amylo process, which have been commercially utilized. A mold fermentation upon a commercial scale, therefore, calls for enormous increases in the surface of the substratum, all of which must be exposed to aeration. This is ordinarily producible by multiplication of

shallow trays filled with some solid or liquid substratum with the necessary ventilation provided, but usually carefully controlled. These requirements increase very greatly the precautions necessary to maintain the cultures sufficiently free from contaminations to yield a satisfactory product. In spite of the difficulties involved, however, it has been found possible to utilize a few of these species commercially by determining and producing growth conditions for desired species at which they seem able to swamp or smother competitive or contaminating species. These conditions may be the optimum environmental conditions for the selected forms or may take advantage of tolerances in these species which insure their dominance under less than optimum conditions.

Even a casual survey of the results accomplished points to many possible lines of future development, since certain species of Aspergillus grow rapidly and cause a wide range of biochemical changes in the substrata. Since few of these fermentations are successfully conducted without involving one or more contaminating species, these applications of Aspergilli to industrial use will be discussed by products or types of activity rather than under the names of the species involved.

ENZYMES OF ASPERGILLI

Waksman (70) reviewed the determinations of enzymes and enzymic activities of microörganisms to 1922. Of the 957 numbers given in this paper, very many refer to the Aspergilli. Knowledge of the enzymes of this genus is seen to be only fragmentary, except that in such collective species as *A. niger* and *A. oryzae*, some members have been intensively studied, and their range of enzymic activity has been shown to be very great. It has also proved possible in a small number of these activities which have been exhaustively studied to intensify the enzyme production greatly by proper manipulation of the composition of the nutrient used or of the conditions of growth or both. Very great possibilities of such development remain untouched.

Aside from the special study of particular problems and the intensive study of certain species, the list of enzymic activities determined for Aspergilli represents incidental information rather than exhaustive investigation, and carries with it no assurance that the measure of a particular biochemical activity reported for certain forms may not be greatly exceeded by that of other and very different species.

A list of enzymes on the following page compiled from the literature, as found in Aspergilli may be of interest (following Waksman (70) for the most part):

Lipase in *A. niger* and in *A. terricola*
Cytase in *A. oryzae*, *A. wentii*, and *A. cellulosae*
Amylase (diastase) in *A. oryzae* and *A. niger*
Inulase in *A. niger*, not in *A. oryzae*
Raffinase in *A. niger*
Gentianase in *A. niger*
Melezitase in *A. niger*
Invertase in *A. niger* and *A. oryzae* groups
Zymase in *A. niger*
Maltase in *A. oryzae* and *A. niger*
Tannase in *A. niger* and *A. oryzae*
Trehalase in *A. niger* and *A. wentii*
Cellobiase in *A. niger* and *A. wentii*
Emulsin in *A. niger*, *A. oryzae*, *A. wentii*, *A. fumigatus*, *A. terricola*, and *A. glaucus*
Urease in *A. niger*
Proteolytic enzymes, such as rennet, in *A. niger*, *A. oryzae*, and probably others
Protease in *A. niger*
Nuclease in *A. niger*
Rennet in many Aspergilli, especially studied in *A. niger* and *A. oryzae*

Such a list of enzyme determinations as that of Waksman is limited to the specific chemical tests calculated to obtain positive evidence of presence under restricted and interpretable conditions. Special study has been given to particular activities of certain Aspergilli in which effects have been demonstrated without enzyme demonstration. In many such reports, the presence of enzymic activity is assumed.

Certain of these enzymic activities will be separately discussed here. Others having become industrially important will be considered in connection with the commercial processes developed.

ACID-PRODUCTION BY ASPERGILLI

Acid production from sugars is common among the species of Aspergillus. The susceptibility of particular sugars; the rate of acidification, kind of acid and total acidity produced varies with the species and with the condition of culture. Falck and Van Beyma (71) report that the black, brown, and light brown Aspergilli produce acid freely, the yellow and greenish yellow forms produce moderate amounts, and white or pale forms produce only slight quantities. In general, any Aspergillus grown upon a substratum containing sugar fermentable by that species produces an acid reaction in the early stages of culture. The nature of the acid produced has been studied only for a small number of species

and a narrow range of conditions. The investigations already reported suggest large possibilities of academic interest with the probability of occasional results of commercial importance from further work.

THE OXALIC ACID FERMENTATION

Wehmer (72) in 1891 made an extended study of oxalic acid production by *A. niger*. This was followed by a series of papers (73). In the course of these investigations, it was clearly shown that *A. niger* (strain not defined more closely) was capable of decomposing sugars with the abundant production of free oxalic acid. Commercially this fermentation was not developed, because oxalic acid can be produced more cheaply by other means. In 1914, however, Currie and Thom (74) resurveyed this problem from the standpoint of measuring the comparative ability of a series of Penicillia and later of black Aspergilli to produce this acid. From a large number of strains in culture, 17 black Aspergilli, one variety of *A. ochraceus*, a purple-brown form tentatively identified as *A. violaceo-fuscus* Gasperini, and an unidentified sclerotium producing purple-brown organism, were selected and tested. Among these strains, the most active strain (no. 142) produced 6 to 7 times as much oxalic acid in fourteen days as the least active black strain, *A. niger* var. *altipes* of Schiemann. In the series, however, it was shown that while the most active strain (no. 142) produced oxalic acid at a rate which followed closely the total acidity of the culture, there was a distinct lag in oxalic acid as related to total acidity in the product of other strains. This led Currie to identify citric acid as one of the intermediate products of this fermentation.

THE CITRIC ACID FERMENTATION OF A. NIGER

Successful commercial development of citric acid production by Currie (75) was accomplished by selecting from the series under experiment the strain (3528.7) of black Aspergillus, in which the lag between total acidity and oxalic acid was greastest and by experimental work, which showed that oxalic acid production could be entirely inhibited by proper concentration of the sugar in the medium and by proper manipulation. Adjustment of these reactions to factory practice followed the line of determining by extensive quantitative experiment the optimum conditions for this function of the mold with as complete as possible suppression of all objectionable features of mold activity. While the detailed factory arrangements have been concealed, certain essential facts are

well-known. The culture medium is carefully adjusted in concentration and quantity to introduce the least possible impurity into the fermented liquor. Refined sucrose has been used, since it furnishes the cheapest fermentable carbohydrate which contains little impurity to interfere with recovery of acid. Further study may alter the process sufficiently to use cheaper material, but involves many difficulties. Aeration, temperature, and length of fermentation period must be so adjusted as to permit the least possible metabolic loss as carbon dioxide, heat, and in mass of mold produced as a waste product. The fermented liquor presents a comparatively simple problem of purification, thus reducing to a minimum the cost of preparing the citric acid for sale. The mycelium must be pressed, leached, and repressed to recover the utmost yield. This process has proved commercially practicable in competition with natural sources of citric acid.

Experimental work covering the field of citric acid production has also been reported by Molliard (76), Wehmer, and in a series of papers by Butkewitsch.

GLUCONIC ACID

Butkewitsch (77) in studying the mechanism of acid production from sugar by *A. niger* confirmed Molliard (78) in finding gluconic acid to be produced along with citric and oxalic acids.

Further experiments indicated this acid to be an intermediate between sugar and oxalic acid, but not to be transformed into citric acid. The gluconic acid was readily precipitated as a calcium salt by using alcohol, but no commercial development of its manufacture is reported.

KOJI ACID

According to Yabuta (79), Saito first noted that when ferric chloride solution is added to cultures of *Aspergillus oryzae* a deep red color is produced. Yabuta isolated from cultures of *Aspergillus oryzae* on boiled rice a crystalline substance responsible for the ferric chloride reaction to which he gave the name koji acid. After a further study of koji acid, Yabuta concluded that it is a derivative of γ-pyrone and this is chiefly interesting because of the relationship between γ-pyrone and the flavones, the latter being the parent substances of numerous plant pigments.

Koji acid reacts readily to form acetyl and benzoyl derivatives and, therefore, contains, at least, one hydroxyl group. It forms salts with various metals, such as copper and lead. The red ferric chloride re-

action is no doubt a typical reaction of the hydroxyl group similar to that of a true phenol. He reported this acid to be produced by *A. oryzae*, *A. albus*, *A. candidus* and *A. nidulans*, but not by *A. okazakii*, *A. fumigatus*, *A. niger*, *A. wentii*, *A. ochraceus*, *A. glaucus*, *A. clavatus*, or any species of Penicillium or Mucor tested.

FUMARIC ACID PRODUCTION

Wehmer (80) has described and patented a fermenting process for the production of fumaric acid. The organism used and named *A. fumaricus* Wehmer is admittedly a strain rather closely related to *A. niger*, but no description has been published and cultures have been refused by Wehmer, although a culture (4668.2) has been contributed by Neuberg under this name. This form has heads reddish or rusty brown rather than purple-brown or black, but resembling in morphology those of *A. niger*. The conidia, although much lighter in color and somewhat larger than the typical size for *A. niger*, have essentially the markings characteristic of the group. Conidial heads in this culture are borne sparingly upon stalks developed upon coarse cottony white masses of aerial mycelium instead of upon a submerged or close lying felt as in the usual form of *A. niger*. No study other than the biochemical discussion by Wehmer has been published, hence the development of this process appears to be limited to those working under Wehmer's German patents.

TANNASE

The gallic acid fermentation. The production of gallic acid from the so-called gall-nuts produced by various species of oak when stimulated by insects has been commercially utilized for a considerable time. These gall-nuts contain high concentrations of tannin compounds. When ground, mixed with water and left to ferment these "nuts" show mold activity restricted to one or very few species, apparently by the high concentration of tannins, which prevent the development of miscellaneous molds and bacteria. Samples examined by us from such a fermenting vat showed predominantly the black Aspergilli (*A. niger*) with minor admixtures of a species of Penicillium (Citromyces) and yeast. It was study of such a fermenting mixture that led Van Tieghem to the identification and description of *Aspergillus niger*. Van Tieghem's (see p. 167) classic studies of this organism in the tannin fermentation form the beginning of our real knowledge of the biochemic importance of the Aspergilli. This work was done before the pure

culture methods now generally used had been perfected, and depended for its soundness upon the ability of *A. niger* to dominate the biochemical activities in such a fermenting mash. In commercial operation, the gall-nuts used come largely from oriental or "near-east" sources. Our own cultural examination of samples from such materials have consistently shown *A. niger* as the dominant species present in the gall-nuts as they are imported.

Knudson (81) studying *A. niger* as fermenting agent for tannic acid found a progressive increase in tannase with the addition of tannic acid in Czapek's solution with 10 per cent sugar. The maximum tannase production occurred, however, when 2 per cent tannic acid had replaced all of the sugar in the formula.

ENZYMIC MIXTURES FROM ASPERGILLI

Investigation of the nature of the enzymes of Aspergilli lagged far behind their practical utilization since the saké fermentation, shoyu making, and numerous other fermentations practiced in the Orient made use of molds of this group as well as mucors, yeasts and bacteria in their empirical production of foods and liquors.

KOJI

These fermentation industries developed the practice of preparing "starters" or masses of rice, soy beans or other material from fermentations giving satisfactory results, for use in the production of successive fermenting mashes or for commercial distribution. The Japanese name koji was applied to such materials with restricting or modifying terms to indicate the character of fermentation involved as saké-koji for saké making, shoyu-koji for the soy fermentation. The use of such products was well established before the identity of the organisms involved was known. Their nature and use was discussed by Kellner (82) and his associates in 1888. These workers determined the various activities of the material as known at that time with quantitative estimation of the enzymic changes induced in laboratory experiments without giving attention to the specific activities of the fungi in pure culture, although *A. oryzae* was already recognized as the most important agent in many of these fermentations.

Koji in its various forms is used in several fermentations to be discussed in ensuing paragraphs.

Following Kellner, Takamine (83) after transferring his work to America, developed his study of the activities of *A. oryzae* and its associates

in the form of many patents for the utilization of *Aspergillus oryzae* (including under *A. oryzae* most of the whole *A. flavus-oryzae* group) in the production of Taka diastase, koji, various food products, and such enzymic mixtures as "polyzime" or the "protozyme" of his competitor. The production of these products is, in general, based upon the use of a cheap, bulky, fibrous, nutrient base, commonly wheat bran in America, or rice bran in Japan, which can be sterilized, moistened to the desired percentage, inoculated with spores of the mold selected and when incubated at favorable temperatures will produce comparatively large volumes of vegetative mycelium. Growth of this mycelium is interrupted at the time of maximum enzymic activity, and the final product manufactured. Such a mass of bran can be dried to inactive condition from which activity can be obtained by moistening or mixing it directly with the material to be affected, or the fresh koji can be ground, extracted, and the solution subjected to such purification as will yield the results desired.

This method of obtaining enzymic material from Aspergillus does not produce a single active principle but an enzymic mixture. Even in the form commercially designated Taka diastase on account of special claims as to amylolytic power, Heuss (84) has called it an arsenal of enzymes, listing proteases, invertase, maltase, sulphatase, phosphatases, inulase, lactase, lipase, as certainly present with evidence that other agents are yet to be identified.

The possibilities of such a mixture were clearly pointed out by Oshima and Church (85), who tested the relative quantities of amylase and protease in 32 strains of Aspergillus, mainly in the *A. flavus-oryzae* group. They report the greatest producer of amylase (loc. cit., table 2) as about 80 times as active as the least; similarly the largest producer of protease developed, at least, 6 times as much enzyme as the least active organism. Between these figures the different races offer a wide variety of forms high in amylase, low in protease; high in protease, low in amylase; high in both or low in both. This work was restricted to two of the many enzymes produced by members of this group. Such enzymic mixtures (86) in more or less crude forms are successfully utilized in the desizing of textiles for which several preparations are commercially available. Proper selection of the mold used in producing an enzyme solution offers many possibilities.

DIASTASE

All Aspergilli as far as reported grow richly upon such starchy media as potato plugs, upon moist cereals, such as corn, oats, wheat or rice.

Diastatic activity is assumed in connection with such growth, but definite studies of diastases have been restricted to a few groups of species.

As an example of the results of special study given to one enzyme, the following review of Funke's (87) study upon *A. niger* is quoted:

This paper contains details of an elaborate series of investigations on the production of diastase by *Aspergillus niger*, especially on the amount produced at different intervals of time and the influence of the medium on enzyme formation.

In determining these points the importance of ascertaining the optimum hydrogen-ion concentration is emphasized. With *A. niger* the optimum range is wide (about pH 5.8 to 3.8), and the organism itself produces sufficient acidity in the standard cultures used to ensure the necessary concentration. The origin of the conidia used for making the test cultures is also important, marked differences in the amount of diastase formed, and other characters appearing when conidia from cultures grown on different media were used. The author recommends the use of conidia grown for two generations on a medium of the same composition as the test cultures.

The results of the tests showed that *A. niger* produces large quantities of diastase when cultivated on a standard mineral nutrient solution with the addition of starch, glucose, or maltose, from about two or three days after inoculation, until a certain maximum amount has been produced, after which no more is secreted during the remaining lifetime of the fungus. The amount produced does not vary according as starch or glucose is present in the culture medium, and though the substitution of maltose delayed the production of diastase, the ultimate total produced was probably the same. The concentration of these substances used is also without apparent effect on the total production of amylase, though higher concentrations than 1 per cent. caused temporary disturbances in enzyme formation, due apparently to the production of substances which inhibit the action of the enzyme. These substances, however, disappear during the course of development, and are without influence on the total amylase ultimately produced.

The diastase formed by *A. niger* is secreted into the culture medium as soon as it is produced, and it may be kept for a long period without losing strength.

The substitution of saccharose for the other carbohydrates mentioned above resulted in a great decrease in amylase production, except when very low concentrations (e.g. 1 per cent) were used. In the latter case the amount was almost as great eventually as with 1 per cent. glucose, but amylase formation did not become considerable until all the saccharose was hydrolysed, and the invert sugar assimilated. Grown on 5 per cent. glycerine, the fungus produced no diastase. On lactose there was no growth, as *A. niger* seems to be unable to form lactase.

As already indicated, members of the *A. flavus-oryzae* group have long been utilized in Japan and China, in connection with certain fermentations. Conspicuous among this is saké, in which only the diastatic

activity of these organisms is significant. This diastatic activity becomes a secondary factor in other fermentations (see Soy), in which protease is the more important agent.

THE SAKÉ FERMENTATION

The diastatic activity of *A. oryzae* is utilized in the saccharification of the starch of rice for fermentation purposes. The earliest workers (88) attributed the alcohol production also to the mold, but yeasts were subsequently proved to produce the alcohol. For this saccharification selected strains of *A. oryzae* with high diastatic power have long been propagated and sold under the name of "tane-koji" or "saké koji" for the making of saké (89) and similar oriental forms of distilled liquor which represent the same general type of product as whiskey from cereals among occidental people. Many different combinations have been developed in which this diastatic action upon rice is made the basis for food or sauce or drink.

SYRUP

The liquor resulting from the diastatic activity of *A. oryzae* upon rice is sometimes concentrated by the Japanese for use as syrup under the name of Mizaume (sweet water). This product is also more or less used in the making of hard candy.

CLARIFYING JELLIES AND FRUIT JUICES

Gums and starchy materials in fruit juices and in the pomace used to provide the pectin necessary in jelly-making produce a turbidity of the final product which lowers its commercial quality. Proteins of various types add to this turbidity. Enzymic extracts (90) of Aspergillus have been found to be effective in dissolving the suspended material to produce a clear liquor after filtration.

In contrast to the study and use of mixtures as such, many investigations have been limited to testing a single enzymic activity of an organism. In such studies the presence of other enzymes in the same solution is disregarded as not affecting the results. Certain of these studies with their possibilities of utilization may be discussed.

CYTASE-CELLULOSE DESTRUCTION

Scales (91) in studying the destruction of plant-tissue in the soil, tested the cellulose-destroying power of a series of Aspergilli. In his experiments, *A. candidus* did not attack cellulose, whereas *A. clavatus*,

A. flavus, A. fumigatus, A. nidulans, A. niger, A. oryzae, A. wentii and *A. terreus* were able to destroy cellulose in a culture medium with ammonium sulphate as a source of nitrogen. Later in the course of studies upon the digestion of cellulose by cattle, Hopffe (92) isolated an Aspergillus which she named *A. cellulosae* and found to be very active in this digestion. A culture under this name received from Neuberg proved to be *A. fumigatus* with the characteristic appearance of the strains common in American soil. No data were presented to indicate actual growth in the alimentary canal; the organism of Hopffe would, therefore, appear to have grown from spores which passed through the digestive tract without destruction.

INULASE

Young (93) selecting the hydrolysis of inulin as a favorable subject found an inulase to be secreted by *A. niger* under all the conditions of culture used. This enzyme was most abundant at the period of sporulation when the metabolic demand would naturally be greatest, and disappeared rapidly after this activity ceased. The presence of inulin in the substratum stimulated the mold to increase the amount of inulase secreted; soluble starch and some other carbohydrates also increased the rate of inulase production more or less definitely in proportion to their relationship in chemical structure to inulin.

INVERTASE

Practically all species of Aspergillus may be grown in nutrient media containing sucrose as the only source of carbon. Interpretation of the mechanism of utilization may be followed through many investigations which have been summarized by Dox (40, p. 30) in his discussion of sucrose, who traces the identification of an inverting enzyme in Aspergilli and Penicillia to Bourquelot (94) followed by Fernbach. Inversion of sucrose appears to precede utilization, hence the presumption follows that invertase (sucrase) is produced by all species capable of utilizing cane-sugar, although special studies of invertase in Aspergilli have been limited to a few species. This inverting activity has been clearly indicated for certain members of the *A. glaucus* group in studies (unpublished) of the losses of soft maple sugar, in which we found that the development of a thin film of mycelium over the surface of masses of maple sugar several inches deep was followed by extensive liquefaction of the mass to a distance of 2 to 3 inches from the mycelium itself. Tests of the syrupy liquid showed extensive inversion. Similarly in

the cane-sugar industry (95) losses are occasionally due to inversion which has been traced more or less consistently to the invertases produced by molds, including several species of Aspergillus. The ability of Aspergilli to attack certain grades of sugar rests upon the tolerance of certain species for very high osmotic pressures, which may include sugar solutions up to 65 or even 75 per cent. Here again a routine determination of water content in a mixed sample becomes deceptive. In calculating the significant concentration of the solution, it is found that the crystals are inactive or inert. Each crystal is surrounded by a film of molasses whose concentration determines whether the organisms present can grow or not. When growth in the molasses film is possible, the invertase produced attacks the sugar crystal, producing a slow inversion and thereby reducing the quantity of sucrose recoverable.

Among the Aspergilli found capable of causing such losses, members of the groups typified by *A. glaucus*, *A. niger* and *A. sydowi* have been most studied. In unpublished experiments by Church and Walek in the Bureau of Chemistry, a 19 per cent solution of sucrose was subjected to the growth of a series of molds for 14 days, then tested for inversion. Two strains of *A. niger*, *N. 0.3* and *Kopeloff 36*, had inverted 67.15 per cent and 79.38 per cent, respectively, of the sucrose; two strains of *A. sydowi*, *Kopeloff 20 and 22*, had inverted 77.01 per cent and 78.02 per cent of the sugar; while an Alternaria had inverted 4.27 per cent of the sucrose in the medium. When the sugar concentration was raised to 30 per cent, the inverting power of the *A. niger* strains was little affected but that of *A. sydowi* dropped to about $\frac{1}{4}$ of that shown in the 19 per cent solution. In practical work, however, Owen (96) regards *A. repens* as the most important species concerned in the deterioration of sugar products. This is consistent with our experience that members of the *A. glaucus* group are the dominant molds in products sweetened to percentages just below the point at which all spoilage is excluded.

INVERTASE SYRUP

The possibility of producing an invert sugar syrup by the use of cultures of an Aspergillus was demonstrated in the Bureau of Chemistry by Brewster (unpublished results) using *A. wentii*. The cultures grown upon bran as used in the production of enzymes from *A. oryzae* proved rich enough in invertase to produce the desired qualities in a syrup. The expense of invertase production from *A. wentii* proved too great to encourage its adaptation to commercial uses under present conditions.

PROTEASE

Growth activities of Aspergilli upon certain organic nitrogenous products have been fairly widely studied. Milk, gelatin, beef-extracts, peptone, egg-albumen, and similar products have been used widely to test their growth promoting or inhibiting power as sources of nitrogen for these species. Gelatin liquefaction is thus shown to be a very common power in this group. Most of the species liquefy gelatin media with the production of an alkaline reaction; *A. niger* and its near relatives produce this liquefaction with a strong and persistent acid reaction.

In connection with his studies of the enzymes of the members of the *A. flavus-oryzae* group in our laboratory, Oshima (97) found that the proteolytic enzymes of these forms can digest native proteins, such as beef muscle, egg white, glycinin, edestin, and casein with the production of amino-acids; the optimum hydrogen-ion concentration for proteolytic action varied with the race of mold used and the substratum. Oshima and Church (98) found that the enzymes, amylase and protease, were produced by all the species tested in this group, but in widely different strengths; out of many strains tested, four strains were found to produce much larger quantities of enzymes than the others; that the strength of the amylase and protease produced was not seriously affected by the stimulating power of the medium; that the intracellular enzymes pass out of the mycelium into the culture medium soon after spore formation, and that the extracellular enzymes continue to increase as long as mycelial growth continues. In testing two strains of *A. ochraceus* obtained in connection with the Katsuobushi fermentation (see fish), Oshima found them both high in protease and low in amylase production.

SOY FERMENTATION

Soy beans as a staple food in China, Japan and adjacent regions are subjected to various fermenting processes with molds as a major factor in the results obtained. Extensive studies of these processes have been conducted in Japan by Teizo Takahashi, Kita and others, and in America by Church, and Church and Oshima. The organisms used in this fermentation belong to the great series discussed by Thom and Church, as the *A. flavus-oryzae* group, and mostly resemble the forms commonly regarded as *A. flavus* more nearly than *A. oryzae*. The soy bean generally used in this fermentation is almost free from starch. It consists largely of protein. For success in soy fermentation, therefore, careful selection among the multitude of strains with the general characters of

the group Aspergillus *flavus-oryzae* has made possible the use of strains in which proteolytic enzymes are produced in rather large amounts and carbohydrate splitting is reduced, although it is probably not in any case entirely suppressed (see Oshima and Church). The beans for this fermentation are cooked, commonly mixed with roasted and ground wheat, inoculated with cultures of the desired strain and incubated until each bean is well covered with the fruiting hyphae of the species. This mass known as koji, forms the basal material of several products differing markedly in final condition.

Efforts are made to confine the organisms involved in koji production as closely as possible to the molds used as an inoculum, but the entire exclusion of other molds and bacteria is not found possible. Mucors, *A. tamarii*, undesirable members of the *A. flavus* group, and bacteria largely of the so-called *mesentericus* and *subtilis* groups are commonly present and may dominate the culture if mistakes are made in the moisture content of the original material or in the temperature or humidity of the fermentation room (koji chamber). As active proteolytic organisms, both the mucors and bacteria found must be reckoned contributory factors in the digestion obtained under many of the commercial conditions of operation.

Soy sauce: For the manufacture of soy sauce, which is widely used throughout oriental countries as a flavoring substance, the koji is immersed in a brine of high sodium chloride content, in which a very slow digestion process is allowed to develop in a period of several months to two or three years. Special types of yeast are believed to play some part in this digestion process. The final product is a dark, heavy salty liquid, with flavors attributed to various factors, but primarily dependent upon the molds of the original koji room.

The finished sauce is used directly or blended with other products. As imported, it is a common component of table sauces used in America and Europe. Experimental work by the United States Department of Agriculture showed that it was entirely possible to transplant this fermentation industry to America, although only a few establishments have thus far developed production upon a commercial scale.

Tamari is another sauce made with soy beans alone or with soy beans and rice for a starchy component, or with other variations. It is made by a shorter fermentation process. Kita believed that where tamari sauce was made empirically it owed its individuality to an Aspergillus, which he named *A. tamarii*. Tamari differs in flavor from soy or shoyu apparently on account of the different digestive products of *A. tamarii* in contrast to the *A. flavus* type of organism.

MISO

Another series of soy bean products under the name Miso (99), with varietal designations is produced by mixing cooked soy beans with an Aspergillus koji (or starter), adding varying amounts of salt and ripening the mixture. The various types of miso resulting vary with the amount of growth of mold (if any) permitted after mixing, with the strength of the brine used, and the flavoring substances added. The enzymes of Aspergilli are in all of these products the principal agents in a fermentation which develops flavor in this highly nitrogenous but comparatively tasteless food so widely used in the Orient.

These soy and cereal products as discussed are only a few of the better known examples of a wide range of oriental food products produced by fermentations in which Aspergilli play a major rôle. Many variations in process with more or less widely known varietal names to the resulting products are described in the extensive literature of this subject. Only a few of these papers can be cited (100).

COFFEE FERMENTATION

Recent patents (101) cover a fermentation of coffee to bring about desired changes in the flavor of the product. In this process, the green coffee was spread in layers several inches in depth, water enough to support mold growth was added and the mass inoculated with cultures of an Aspergillus isolated from coffee of desirable flavor. Samples of the fermenting product obtained and studied by us showed the organism used, to be a strain of *A. ochraceus* not morphologically separable from Wilhelm's species. Further observation of lots of coffee subjected to the conditions prescribed for fermentation under this patent showed strains of *A. niger*, *A. tamarii* and *A. flavus* to be also capable of developing. Bacterial activity did not seem to be great, apparently on account of the presence of inhibiting substances (102), which also restrict the molds developing to very few species. The promoters of the fermentation after experimenting with *A. niger*, report that *A. ochraceus* alone of the species tried gave satisfactory results in flavor. No chemical study of the changes induced has been made public.

FISH FERMENTATION

The Japanese have a series of fermented preparations made from fish, in some of which molds are the active agents in producing the qualities desired. Among these the product known as Katsuobushi (103) has

been most fully described by them. In this product fish of the tuna group is cut into strips, cooked, packed in masses and fermented, then dried for commercial distribution. Yukawa (103) has described *A. gymnosardae* as an essential agent of this fermentation in terms that ally his species with the *A. flavus-oryzae* group, perhaps approaching *A. pseudo-flavus* Saito. He described *A. melleus* (belonging to the *A. ochraceus* group) as a contaminating organism. The descriptions of this fermenting process indicate great variability in practice and in the uniformity of the agents found. At best other species of Aspergillus are recognized as present. Yukawa describes one of these as *A. melleus*. A culture received under this name from Hanzawa and corresponding fairly well with Yukawa's description proved to be a member of the *A. ochraceus* group. Similarly starters for this fermentation collected and contributed by Oshima (personal communication) proved to be strains of the same group, although members of the *A. glaucus* group were also found. In examining various samples of Katsuobushi as obtained in America, however, we have also found perithecia of members of the *A. glaucus* group abundantly, commonly readily visible in the cracks and seams of the broken masses. This tends to confirm the conclusion (104) of Hanzawa and Kita that members of the *A. glaucus* group are the principal molds present in this fermentation. Whether the *A. glaucus* type of mold is the only active factor in the ripening of these fish products remains unsettled, but the distribution of the perithecia throughout the mass in the specimens examined point to entrance and development early enough in the preparation to allow extensive mycelial growth antecedent to the production of the fruiting organs observed. Such growth could hardly fail to be a factor in the final condition of the ripened product.

MOLDY NUTS

In the spoilage of nuts, Aspergilli become, perhaps, the most frequent and conspicuous molds encountered. Nuts, as a rule, are very low in water content and very high in fat or oil. At the concentrations common in walnuts, filberts, chestnuts, Brazil nuts and similar products, the members of the *A. glaucus* group are very abundant in both conidial and ascosporic form; *A. flavus* and even *A. oryzae* have been found; *A. wentii* and *A. tamarii* are occasionally seen; the other species are less common or absent.

ONIONS INFECTED BY ASPERGILLUS

Extensive infection of the outer scales of the onion by *A. niger* is frequently observed. Van Pelt (105) reported this mold as obtaining entrance through torn, bruised or broken places in harvesting the onion, not as a parasite of growing onions in the field. After once gaining a foothold, *A. niger* may be confined to small black spots merely injuring the appearance of the crop or invade many layers of scales destroying much tissue and injuring the market value of the crop. In studies of onion seed, Van Pelt found spores of *A. niger* upon 50 per cent of the seeds, but did not prove direct infection of the growing plant. It is primarily a disease of the onion during the storage and marketing process as indicated by reports from the market inspection service (1918) that in one terminal market an average of 18 per cent of the onions handled during a single period showed infections of *A. niger* "ranging from small spots to the entire surface of the onion." As we have seen these moldy onions, the infections consisted of *A. niger* very nearly, if not quite, in pure culture. Saccardo in Fungi italici 903, 1881, found black Aspergilli upon onions and identified his material as *A. phaeocephalus* Dur. and Mont.

Walker, Lindegren and Bachmann (106) regard *A. niger* as less important upon onions than another Aspergillus described in this book as *A. alliaceus* n. sp. resembling the *A. ochraceus* and *A. sulphureus* series in superficial details, but producing numerous sclerotia and few ochraceous heads (no. 4660) and placed in the Key in a section near *S. quercina* Bainier. Inoculated into onion bulbs, this species proved to be an active parasite.

TOBACCO MOLDS

Aspergilli have been frequently reported in connection with the molding of tobacco. Ruhmwerth (107) found *A. niger* upon tobacco in Hungary and capable of reducing tobacco leaves to black powder. We have found the same species several times upon tobacco submitted to us as moldy. Molding of cigars by *Aspergillus fumigatus* is reported by Sartory (108). True (109) in studying moldy cigars attributed moldiness rather to the paste used in sealing on the wrapper than to direct molding of the tobacco itself and reports the trouble to be controlled by making the paste with a boric acid solution (1 ounce of boric acid to $1\frac{3}{4}$ pints of water) instead of water. Upon those cigars, True reports *A. candidus*, *A. subgriseus* Peck, and *S. castanea* Patterson (= *A. niger*

var.). More recently Shear has submitted to us, cigars molded with an Aspergillus grayish or whitish in appearance and thinly scattered over the surface. This species probably represents the one reported by True as *A. subgriseus* Peck, but fails to grow readily at low concentrations of media, grows freely in 50 per cent sugar, in which it becomes a gray green form with the appearance and approximately the measurements of *A. penicilloides* Speg. belonging near the *A. glaucus* group but without producing ascospores. Some cultures made from the same cigars, however, showed perithecia also, indicating the probable presence of two members of the group rather than a culture of a single species. Careful study of the cigars themselves suggest that the mold flora actually present consisted predominantly of the *A. penicilloides*-like form and that other species obtained in culture represented spores present, but not active in the tobacco. Inoculation experiments in sterilized tobacco show various species capable of growing when the conditions of a pure culture are presented.

LEATHER

Aspergillus niger appears as a serious destructive agent in the tanning industry (110). The tolerance of this species for tannin solutions has long been known. More recently discolorations causing large financial loss in finished leather have been shown to be due to the growth of colonies in the hides during the handling processes, especially where hides are stacked for curing purposes between steps of the tanning process. The changes induced in the texture of the skins are great enough to produce areas which respond differently to the tanning liquors and coloring substances used; permanently discolored areas are thus produced with consequent loss of value in the leather.

CHAPTER VII

Aspergilli and Animal Disease

Aspergilli are frequently encountered in the study of human and animal diseases. Some of the species found have been described as new and specific to the diseases under investigation; others have been reported under names already in use for organisms more or less well-known as saprophytes. In this way, more than 60 names of species of Aspergillus are encountered in the literature of pathology. Among the human cases reported, otomycoses as covering a whole range of affections of the external ear, predominate, followed by skin lesions under the name dermatomycoses, including epidermal and subdermal growths, abscesses and ulcers classed as mycetomas, and by mycotic affections of the air passages commonly referred to as Aspergilloses. Occasional descriptions of Aspergilli, in connection with generalized lesions, stomach and intestinal contents or feces, direct attention to the possibility of other mycotic affections of man. Domestic mammals are subject to similar infections, but the cases have been less carefully reported. Aspergillosis of the air passages is frequently reported in birds. Certain species have been described as attacking insects.

The identification of the species involved in these pathological reports is commonly very unsatisfactory. Some of the forms described as new were certainly members of groups already more or less well described; others named from the literature were probably quite different from the original forms described under the names used. Correct naming of such forms calls for large acquaintance[1] with molds and mycological literature.

The culturing of microörganisms by those trained in medicine has commonly been carried out on media devised for pathogenic bacteria. Such media contain animal substances rather than the carbohydrates which are especially favorable for mold growth. A mold may grow on the substances of the animal body as a pathogenic organism or as a secondary factor in a pathological condition, but in such an environment it commonly either does not develop its fruiting structures at all or

[1] To name as a *new species*, an Aspergillus *new* to the describer who is not a student of molds has been called taxonomic "wickedness" by a distinguished correspondent.

develops such structures differing in form and color from the usual saprophytic cultures upon which our records and descriptions have been drawn. In such lesions, conidial apparatus, if present at all, is very much reduced; vegetative hyphae in some species are very slender and delicate, in others become large, vesiculose or even tend to break up into single cells. However uniformly such structures may be produced, comparative culture under the conditions favorable to the usual morphology of Aspergillus, is necessary before we can place such species correctly within the group. Although certain species of Aspergillus have been studied and described only as found in pathological materials, hence are unknown in culture, most of the forms isolated from such sources have assumed the structures characteristic of saprophytic species when transferred to culture media rich in fermentable carbohydrate.

The literature of mycotic diseases is widely scattered in medical and bacteriological periodicals. Several authors have, however, brought together these descriptions into the form of manuals largely without critical comment or comparative study of the organisms involved (111).

In the literature of pathology, the discussion of mycotic diseases is commonly roughly divided into certain sections which may be presented separately.

OTOMYCOSES

Molds in connection with ulceration of the external ear began to be recognized early in the 19th Century. The earlier reports (112) recognized the agents as molds, but did not attempt the identification of species or varieties of ulceration. The examination consisted in the removal of obstructing masses from the external auditory canal, and the study of the material removed. The reports of such examinations include recognition of gross carelessness which permitted the accumulation of cerumen and filth in the ear, followed by the development of lesions of varying intensity. The materials removed were examined microscopically, commonly after immersion in alcohol or other preservative for varying periods with or without careful handling. All of the reports must be considered and interpreted in the light of the methods of work available at the time. Culture of the organisms involved and confirmative inoculation of laboratory animals were developed much later in the 19th Century.

Examination of these reports shows clearly that many species of mold can develop in the external ear when it is partly or completely

blocked. Frequently several species were found and figured together under one name, with little data for the identification of any one of them as the irritating agent. Among these figures and descriptions, however, it is readily possible to recognize members of certain of the common groups, which have been shown by later investigations to become active agents of disease, and members of other groups whose presence was probably incidental only or very doubtfully significant.

The outstanding organisms of these otomycoses are probably the black Aspergilli which are commonly grouped together as *A. niger* and attributed to Van Tieghem (1867), although an earlier recognizable description from a pathological condition in the human ear is *Sterigmatocystis antacustica* by Cramer (1859). *A. nigricans* of Wreden (1867) was probably one of this group, but is not more closely identifiable. Many such cases have been identified to the group, notably one by Stout (113), whose culture is in our collection and typical in morphology with Van Tieghem's species. Several similar isolations have been made by members of the United States Public Health Service and the cultures forwarded to us for examination. In these cases there was distinct ulceration, often persistent for comparatively long periods under conditions which clearly indicate that some, at least, of these black Aspergilli once established in the ear are capable of maintaining and extending such lesions. Whether *Aspergillus niger* under ordinarily hygienic conditions can originate ulcerations of this kind remains to be proved. Spores of members of this group are common components of dust and dirt, especially abundant where grain and many other types of vegetable matter are handled, hence are probably a common contaminant of the external ear. Ulceration by *A. niger* is, however, only occasionally encountered, hence the probability that such mycotic infections are secondary rather than primary is clearly suggested.

Another series of cases recorded in the European literature appears to be caused by *A. fumigatus* in some of its many varieties. Among the names probably to be included in this group are *A. nigrescens* Robin, *A. malignus* Lindt, *A. hageni* Hallier, and *A. nölting*. *Aspergillus fumigatus* is probably the most dangerous pathogenic mold of the group, hence may be considered a probable cause of such disease. It is, however, equally, if not more abundant, in the soil and in the dust produced in handling agricultural products than *A. niger*, but isolations from the ear are not common. It is, therefore, entirely probable that *A. fumigatus* may be only a secondary parasite in such situations developing only if the lining of the auditory canal is already broken or irritated.

Of the other species reported, Moquin-Tandon used the name *A. auriculaire; A. nidulans* appears to occur occasionally in the ear as well as in skin lesions; and *S. hortai* has been described from a case in Brazil. The *A. flavus* group is represented in the literature of otomycosis by certain identifications of *A. flavescens* Wreden (1867), and by *A. siebenmanni* of Costantin and Lucet. Sartory states that *A. repens* is found in 8 per cent of all the cases of otomycosis and that *A. herbariorum* is occasionally found.

There is little doubt as to the occasional finding of Aspergilli within the external ear, but the record of inoculations in the laboratory leaves a serious doubt as to primary responsibility of any of them, although such forms as *A. niger* are clearly able to maintain and probably occasionally to extend an ulcerated area.

DERMATOMYCOSES AND MYCETOMAS

The occurrence of certain of these Aspergilli in lesions of the skin and flesh near the surface of the body is well authenticated. Wehmer described *A. tokelau* as it was found in preserved material from such a case. Similarly, *A. bouffardi* was found in a "black mycetoma," in the actual sections from the diseased tissue. *A. barbae* from the beard of African patient by Castellani; *A. pictor* R. Blanchard from the lesions of so-called Caratés. On the other hand, *A. nidulans* in one or more of its varieties has been grown from cultures from massive growths (mycetomas) of various kinds; *A. fontoynonti* Guéguen from joint affections; *A. gratioti* Langeron and *A. jeanselmeiota* from infected nails called onychomycoses by Sartory (114), and *S. tunetana* from an ulcer on the hand. Extreme difficulty has been encountered in the interpretation of these findings. Few of those who have studied the actual diseased tissue had critical knowledge of molds or of the cultural methods involved in their identification, while such animal inoculations as have been tried have very commonly given negative results. The exact relation of these species to the pathogenic conditions in which they were found remains, therefore, to be proved by further study (115).

PULMONARY LESIONS—HUMAN[2]

A series of Aspergilli have been isolated from diseases of the air pas-

[2] Recent workers have found in appreciable numbers, lung lesions clinically diagnosed as tuberculosis but in which the causal agents appear to belong to the nearly related group, Penicillium.

sages. As described, these molds mostly agree in essentials of morphology with *A. fumigatus* as described by Fresenius in 1863. The cultural differences observed probably account for the use of various names, such as *A. bronchialis* Blumentritt, *A. lignieresi* Costantin and Lucet, *A. pulmonum hominis*, *A. ramosus* Hallier, for cultures obtained in different lands or under different clinical conditions.

Aspergillus fumigatus has been identified in pulmonary lesions from cattle and swine occasionally in Europe and by the veterinary pathologists of the United States Department of Agriculture. These cases, although, as a rule, isolated, are recorded from widely separated regions and clearly reliable. This species must, therefore, be recognized as capable of attacking the domestic mammals and occasionally man, although the conditions necessary to infection are not understood (116).

In addition to members of the *A. fumigatus* group, Mirsky described *A. versicolor* from the sputum of a pulmonary case, and Sartory and Flament have described *A. mencieri* as isolated from sputum of a patient with lung trouble. This species belongs to the *A. glaucus* group and is described as producing the characteristic perithecia and ascospores of that group regularly, but did not show pathogenicity in their experiments with animals.

The extensive use of members of the *A. flavus-oryzae* group in the saké and shoyu fermentations in the Orient furnish an extensive test of the possible pathogenicity of these species. Specific inquiry among Japanese students of these fermentations shows no knowledge of the prevalence of infections by *A. flavus* or any member of the group in the ear. Oshima (personal communication) found one manufacturer who reported more or less vaguely defined irritation of the respiratory organs whether from infection or from the irritation due to inhaling conidia as dust remains undetermined. One day in such a factory produced great irritation in one of us apparently limited to the irritant effect of the spores as dust, since no persistent evidence of mold activity was experienced.

BIRDS

Since aspergillosis of the respiratory organs is very common in birds, the molds involved must be considered together with those encountered in mammals. Peck described *A. aviarius* from the air sacs of a canary, but in terms closely resembling *A. fumigatus*. *A. fumigatus* has been reported as a cause of death in birds in zoological gardens frequently in widely separate countries, but usually without attempts to define

varieties of the species. In studying one outbreak, the veterinary pathologists of the United States Department of Agriculture found that the chicks involved had been bedded with moldy straw and had all developed aspergillosis of the lungs, due to *Aspergillus fumigatus*. On removal of the moldy straw and the substitution of clean bedding, this epidemic subsided. We have isolated it several times from domestic fowls at a time when a considerable number of hens died in a single establishment. These cases led to our isolation of apparently the same strain from the soil of the newly built pens which extended into a swamp area containing large quantities of decaying vegetable matter. Since that time hundreds of strains from studies of soil, from cereals, and many other sources have been examined, and have led to the conviction that Fresenius' description fixes certain essential characters found in a large group of strains, varying in minor cultural characters and in biochemical activity, but difficultly distinguishable by any morphological description. Costantin and Lucet (111) after describing certain species, summarized their knowledge of the group on the basis of the pathogenicity of the forms studied, toward laboratory animals, especially rabbits and hens.

INSECTS

Parasitism of insects by fungi is well authenticated, but the number of Aspergilli identified in such cases has been small. Montagne (1849) described *A. fulvus* from silkworms, but the species has not since been recorded. Von Höhnel described *A. citrisporus* from the excrement of certain larvae. This organism was again found by Thaxter, whose strain is No. 4186.10 in our collection. It has also been identified by Kauffman in Michigan. We have seen his specimen in the collection of the Michigan Academy of Science. Speare working in Hawaii described *A. parasiticus* as causing a disease of mealy bugs of sugar cane. The type culture appears in our collection as No. 3509. An organism indistinguishable from No. 3509 was isolated here from mealy bugs of sugar cane obtained for us from Demerara by J. R. Johnston and another culture in our collection was isolated in his studies of the sugar industry of Louisiana by Kopeloff. Another member of this group has recently been isolated from corn borers in New Jersey. Some inoculations of mealy bugs by Johnston (personal communication) indicated, however, that pathogenicity to mealy bugs is not limited to *A. parasiticus*, but may be fairly common among other strains of the *A. flavus* group to which *A. parasiticus* belongs. An organism of this same group parasitizing bees has been discussed by Vincens (117) as producing a

disease widely distributed through Europe and causing losses, but reported as fairly easily controlled by proper handling. In America, Burnside (personal conference) has found certain strains of *A. flavus* as definitely destructive organisms when introduced into healthy hives of bees with *A. ochraceus* occasionally causing loss and other species of Aspergillus less commonly seen but without definitely proved relationship to the troubles encountered.

Certain experiments by Galli-Valerio and Rochaz-de Jongh (118) indicated that species of *A. glaucus* (?) and *A. niger*, if added in quantity to water containing larvae of the common mosquito genera Culex and Anopheles, could lodge and grow in these larvae causing death. Closer survey of the diseases of insects may be expected to increase the number of Aspergilli involved.

PELLAGRA

From their epidemiological studies, many investigators, especially in southern Europe, reached the conclusion that moldy corn (maize) was the cause of pellagra. In testing this hypothesis very many studies of the molding of corn and corn products were made principally between 1895 and 1915. This literature was reviewed rather fully by Alsberg and Black (119) in 1913, but since the experimental results were largely negative, while pellagra appears to be controllable by dietary methods, work upon this line has been largely abandoned. Jobling and Arnold (120), however, in studying a series of cases found strains of the *A. glaucus* group in the feces of pellagrous patients. Their strain produces strong fluorescence under culture conditions, as already reported by Kloecker (121). This caused them to direct attention again to the photodynamic effects of such organisms as a possible factor in this disease. Whether spoiled food has any significance in these cases remains unproved therefore.

PATHOGENICITY

Much experimentation with rabbits, guinea pigs and dogs is reported, principally by the French mycologists. Unfortunately a large part of this work has been inconclusive. Organisms taken directly from lesions have failed to produce disease in experimental animals; other organisms never reported as the cause of actual disease were found able to produce lesions and deaths when injected into laboratory animals. In a general way, any species growing well at blood-heat (37° to 38°C.) has been regarded as possibly pathogenic. Positive results in an infec-

tion experiment were, therefore, confirmatory of this hypothesis while failure in such an experiment did not exclude pathogenicity under other conditions. Limitation of pathogenicity to particular species of host is only a partial explanation. One case may be described from the personal experience of one of us. In operating a breast pump upon an inflamed mammary gland, it was noted that streams of milk were interrupted by what appeared to be clots or plugs, which floated as little "islands" of pin head size, on the surface of the milk in the reservoir when dislodged by heavy suction. When examined with the microscope these proved to be colonies of Aspergillus with green conidial heads fully developed. When transferred to culture media these grew as colonies of a strain of the *A. versicolor* group (3512), which, however, refused to grow in the incubator at 37°C., although they had grown and fruited in a tightly bound breast under irritation, which must have been associated with fully normal blood temperature. No further development of the infection was noted, although the strain isolated by Mirsky and originally described as *A. versicolor* was obtained from sputum of a tuberculous patient.

Further experimentation will be required to determine the method of infection and to measure the responsibility of species of Aspergillus for the lesions in which they are occasionally found. Certainly some of them are active agents of disease after they become implanted in particular tissues. It is entirely probable that some of the species reported were only accidentally present as inactive spores or as saprophytes in the lesions from which they were isolated or in which they were seen with the aid of the microscope.

ASPERGILLI UPON MISCELLANEOUS ANIMAL PRODUCTS

Two species have been reported from eggs, *A. glaucoides* Spring and *A. heterocephalus* Spring. In studies of stored meat, we have encountered members of the *A. glaucus* group as very abundant, if not the principal mold observed, although various species, *A. candidus*, *A. ochraceus*, *A. niger* appeared as isolated colonies. Upon fresh meats, these Aspergilli produce conidia only, no yellow perithecia and ascospores are found. Carbone in studying Italian sausages described *A. belfanti* and *A. tiraboschii* as characteristic species upon these products. Although these species have not been identified by us, *A. belfanti* appears by description to be allied with the *A. glaucus* group, and *A. tiraboschii* to be near *A. versicolor*, which we have frequently observed on very old and very moldy salted and dried meat products.

MOLDY FOOD AND FEEDING STUFFS

In studying "forage poisoning" of horses, Haslam (122) fed selected corn, including lots showing *A. flavus* and *A. niger* among other molds with positive toxic effect, but when he fed cultures made under controlled conditions in the laboratory, no poisoning resulted. This report is characteristic of many efforts to determine what relation, if any, molds bear to toxicity in foods and feeding stuffs. In these experiments occasional poisoning resulted from feeding the moldy product, but when care was taken to limit the decomposition present in the mass by pure culture methods to the effects of a single mold, results were so nearly all negative as to leave the few positive reports subject to question until confirmed. There exist nevertheless enough positive results such as the feeding of grain molded by *A. maydis* (Quevedo) to support the advice to exclude moldy products from human and animal consumption.

Church and Buckley (123) in feeding laboratory animals used the following Aspergilli: *A. terreus, A. tamarii, A. nidulans, A. flavus,* and later (unpublished records in the Bureau of Chemistry) Church and Giltner fed grain (oats) to horses and sheep after sterilizing the grain and inoculating with pure cultures of *A. niger* (142), *A. flavus* (4010.8), *A. fumigatus* (118), and *A. tamarii* (4010.2). These experiments, although continued over four to six week periods, failed to show evidence of toxicity in the feed itself or of infectivity due to the spores which filled the air which the animal breathed. In these studies, care was taken to sterilize the oats before inoculation, since grain under natural conditions is commonly heavily contaminated with bacteria which undoubtedly occasionally include toxicogenic species, such as *B. botulinus*. The negative results obtained do not exclude the possibility of poisonous by-products from fungous activity, but agree with many other results in limiting the probability of such findings. Moldy feed usually carries extensive bacterial infection, hence the possibility of symbiotic effects and of injury from bacterial poisoning or infection is open. Exactly the same conclusion arises from all our studies of human food. Jörgensen (124) is probably correct in interpreting molds as the conspicuous but usually harmless causes of damage or decay in which they are accompanied by bacteria or other organisms not visible to the naked eye, yet which may be dangerous. The presence of mold in foodstuffs is, therefore, a clear warning of unsoundness with its possibilities of danger.

The list (on pages 86 and 87) gives the names of species reported in connection with lesions or disease of man or other animals without

necessarily indicating that these species are responsible for the pathological conditions described. For convenience the list is arranged alphabetically and the general nature of the infection indicated. Species whose affinities are known are described in connection with the proper groups as indicated; species not sufficiently described for grouping are discussed as fully as possible in pages 85 to 90.

The following species reported as pathogenic are not assignable with reasonable probability to any of the groups described in the taxonomic chapters. They are arranged here for convenience in alphabetical order of specific names.

S. aurea Greco. (Greco, N. V. Origine des tumeurs et mycoses Argentines. Buenos Ayres 1916, pp. 671–694, and figures 418 to 428.)

This Aspergillus developed in some of the cultures from autopsy material of a case showing extensive ulcerations complicated with syphilis. Photographs of lesions and histological preparations are given. The presence of mold hyphae in the lesions was fully established, but the inoculations into rabbits as reported were not very convincing as to pathogenicity or, perhaps, the identity of the parasite. Since no other isolation of this species has been reported, its significance as a human parasite must remain for the present problematical.

The following descriptive data are extracted from the discussion on pages 684 and 685. Stalks 6 to 10μ in diameter, with walls thick, yellow, and so described as to suggest a misinterpretation of the granulations and pitting of the *A. ochraceus* wall; heads golden yellow to brick red (! CT), 70 to 80μ in diameter; vesicle more or less globose, about 20 to 30μ in diameter; sterigmata, primary up to 30μ long, 4 to 5μ in diameter at base and 10 to 12μ at apex, secondary 8 to 10 by 2 to 3μ; conidia nearly globose 3 to 4μ. By the terms of this description, this species is close to the yellow series in the *A. ochraceus* group. It has not been seen by us.

A. auriculaire Moquin-Tandon. Éléments de botanique médicale. 2. éd., p. 466. 1866. This name was not accompanied by adequate description to separate the form intended from other species which are occasionally found in the ear.

A. barbae Castellani. Cited by Sartory, A. Champignons parasites de l'homme et des animaux, p. 595. 1922. This organism was found in the beard of a native of Uganda and again from Ceylon. The only further information given is conidia 4 to 5μ, globose, dark brown.

A. bouffardi Brumpt. (Brumpt, E. Les Mycétomes. Archives de Parasitologie 10: 489–572, pls. XII to XXI. 1906; diagnosis p.

Alphabetical list of Aspergilli reported under conditions which suggested pathogenicity to the observer*

NAMED USED	GROUP RELATIONSHIP	PLACE REPORTED AS PATHOGENE	PAGE
S. aurea Greco	*A. ochraceus*	Generalized septicemia	85
S. antacustica Cramer	*A. niger*	Ear	176
A. auriculaire Moquin-Tandon	? not described	Ear	85
A. aviarius Peck	*A. fumigatus*	Coctal area of canary	135
A. barbae Castellani	Probably *A. niger*	Beard of African	85
A. bouffardi Brumpt	Not cultivated	Foot-mycetoma	85
A. bronchialis Blumentritt	*A. fumigatus*	Lungs, bronchi	134
A. candidus Link	? identity	Air sacs of a bullfinch	157
A. carbonarius? Bainier. De-Meis and Parascandolo	*A. niger*	Throat of a diphtheritic; contaminant?	181
A. citrisporus Von Höhnel		Insects	191
A. condylomatae Greco	Not described		88
A. desseyi Speg.	—?	Human dermatosis	88
A. dubius Corda	?–? identification	In lungs of flamingo	160
A. flavescens Wreden		Ear	141
A. flavus Link	*A. flavus-oryzae*	Ear	198
A. flavus Link	*A. flavus-oryzae*	On bees	198
A. fontoynonti Guéguen	*A. glaucus* group	Abscess	115
A. fulvus Mont.	?	Silk-worm	195
A. fumigatoides Bainier and Sartory	*A. fumigatus*	Pathogenic for rabbit and guinea pig	131
A. fumigatus Fresenius	*A. fumigatus*	Lungs, common in birds, occasional in man, cattle, horses, etc. Reported also in kidneys, in skin, in the cornea, in the ear, and in the viscera	129
A. fumigatus var. *minimus* Sartory	*A. fumigatus*	In sputum of a pulmonary case	133
A. fumigatus var. *alpha* Sion and Alexandrescu	*A. fumigatus*	Experimental study only	133
A. fungoides Greco	?	A mycosis	88
S. fusca Sartory and Jourde, not Bainier	*A. tamarii*?	Laboratory experiment pathogenic to rabbit	194
A. glaucoides Spring	?		136
A. glaucus Link	*A. glaucus*	Ear	101
A. gratioli Sartory	Near *A. fumigatus* and *A. calyptratus*	About the nails. Ulcers on hand	150
A. hageni Hallier	?	Ear	89
E. herbariorum Wiggers	*A. glaucus*	Reported in one case each of rhinomycosis and acid vomiting. Mosquito larvae	106
S. hortai Langeron			152
A. jeanselmei Ota	*A. nidulans*	Onychomycosis	140
A. keratitis Ball		Cornea ulcerated	89
A. lignieresi Cost. and Lucet	*A. fumigatus*	From a penguin, pathogenic to rabbit and fowl	134
S. lutea Bainier? (Sartory and Jourde)	*A. flavus* probably	Pathogenic to rabbit	207

NAME USED	GROUP RELATIONSHIP	PLACE REPORTED AS PATHOGENE	PAGE
A. malignus Lindt	*A. fumigatus*	Ear	132
A. maydis Quevedo	*A. glaucus* group	Poisoning of horses	89
A. mencieri Sartory and Flament	*A. glaucus*	Sputum of lung case	109
A. microsporus Böke		Ear	89
A. micro-virido-citrinus Cost. and Lucet	*A. flavus-oryzae*	Pathogenic for rabbits	205
A. nidulans (Eidam)	*A. nidulans*	Ear	138
A. nidulans form. *cesarii* Pinoy	*A. nidulans*	A mycetoma	140
A. nidulans var. *nicollei* Pinoy	*A. nidulans*	Abscess or mycetoma in Tunis; pathogenic for rabbits	140
A. niger Van Tieghem	*A. niger*	Ear, occasionally reported elsewhere	167
A. nigrescens Robin	*A. fumigatus*?	In a pheasant	135
A. nigricans Wreden	*A. niger*?	Ear	174
A. nölting Hallier		Ear	205
A. ochraceus Wilhelm	*A. ochraceus*	On bees	188
A. oryzae var. *basidiferens* Cost. and Lucet	*A. flavus-oryzae*	Pathogenic for rabbits only	199
A. parasiticus Speare	*A. flavus-oryzae*	Insects	203
A. penicilloides Speg.	*A. glaucus* group	Listed by Sartory but probably not pathogenic	126
A. pictor R. Blanchard	?	Skin lesions, Carate	90
A. polymorphus Moquin-Tandon	?		90
S. pseudonigra Cost. and Lucet	*A. niger*	From sick horse, probably not pathogenic	175
A. pulmonum hominis Welcker	*A. fumigatus*	Lung	134
Otomyces purpureus Wreden	Doubtful	Ear	
Burnett (preparation seen)	*A. versicolor*	Ear	
A. ramosus Hallier	*A. fumigatus*?	Ear	135
A. repens DeBary	*A. glaucus*	Ear	113
A. rubens Green	?	?	90
A. siebenmanni Cost. and Lucet	*A. flavus*	Ear	206
A. stercoreus Sacc.	?	Feces	122
A. subfuscus Johan-Olsen	Near *A. niger*	To rabbit, intravenous injection	167
A. tokelau Wehmer	*A. glaucus*?	Ulceration	115
S. tunetana Langeron	*A. versicolor*	Ulceration of hand	145
S. versicolor Vuillemin	*A. versicolor*	From sputum of a consumptive	142
A. virido-griseus Cost. and Lucet	*A. fumigatus*	Pathogenic for rabbits (injected in veins)	135
S. unguis Emile-Weil		About the nails	140
A. wehmeri Cost. and Lucet	*A. flavus*		204

* List summarized largely from Sartory, A. Champignons parasites de l'homme et des animaux. Paris, pp. 551–610. 1922. In these pages the author cites the material from many sources, including some species for which no pathogenic reports are available.

532; discussion pp. 525 to 533.) See also Bouffard. Du Mycétome à grains noirs en Afrique. Ann. Hyg. Med. Coloniale 8: 579–590. 1905. Bouffard described a single case of mycetoma of the foot in an African. This case was entirely cured by surgical operations which furnished the material described later by Brumpt. No cultures were made and no other case has been found in the literature. The following description is rearranged from Brumpt's discussion and diagnosis:

Colonies forming helicoid granular masses imbedded in the flesh, with the outer or cortical layer brown pigmented, and apparently inactive, growing only at one end of the spiral which remains uncolored; with conidial production in the central area only; mycelium silvery white when young, in the peripheral layer of the pathological mass forming a brown cortical zone; conidiophores erect, unbranched, unseptate, colorless, about 2μ in diameter, bearing heads (vesicles) claviform 4.5μ in diameter by about 6μ; sterigmata not mentioned; conidia 1.3 to 2μ in diameter, globose, smooth, colorless, in chains; chlamydospores globose when terminal 5 to 10μ in diameter, brown, when intercalary colorless.

The description given by Brumpt is inadequate to place this species even as an Aspergillus, although it may be adequate to identify other similar cases. Until, therefore, this species is rediscovered and cultivated, its real affinities will probably remain doubtful.

Aspergillus condylomatae Greco. (Greco, N. V. Origine des tumeurs et mycoses Argentines. Buenos Ayres 1916, pp. 502–504, fig. 295, 296.)

Greco discusses without culture, the mycotic structures encountered in lesions clinically designated "Condylomata acuminata." Neither the figures nor descriptions given identify the organism as an Aspergillus or form any basis for the establishment of a species of Aspergillus.

A. desseyi Speg. (Spegazzini, C. Un nuovo Aspergillus patogeno. Physis (Rev. Soc. Argentina Cien. Nat.) VIII: 115–117, 1 figure. 1925); review only seen, Rev. Appd. Mycol. 4: 542. 1925.

Spegazzini described this species from a human dermatosis found in Buenos Aires. It is reported as highly virulent to rodents, but innocuous to fowls; without sclerotia and without perithecia; with heads bearing conidial chains only in the upper portion of the enlarged or vesicular area; "without differentiated sterigmata larger than the true conidia;" with conidia globose, smooth, smoky in color, and borne in chains of 4 to 6 elements.

Aspergillus fungoides Greco. (Greco, N. V. Origine des tumeurs et mycoses Argentines. Buenos Ayres 1916, pp. 505–509, figs. 297, 298, 299.)

The photographs and descriptions given indicate a mycotic infection, but give no ground for the creation of a new species of Aspergillus for the organism. Until Greco succeeds in obtaining the necessary material to complete the study and description of this form the name is practically a *nomen nudum.*

A. hageni Hallier. Cattaneo Mico. Corp. Um., p. 123, pl. 6, fig. 8. Florentin, 1892; syn. Otomyces hageni Hallier, Zeitschr. Parasitol. I: 195. 1869; and 2: 22, 233, and 259, pl. 5. 1870. In the latter article the descriptions are inadequate, while the figures given include under Otomyces hageni, fruiting hyphae which evidently represent Mucors, Penicillia, and probably, at least, two species of Aspergillus.

A. keratitis Ball. Amer. Med. 2: 31. 1901. This organism was found in an ulcer in the human cornea. No adequate description was given.

A. maydis Quevedo. Estudio de un Aspergillus pathogeno. De Agronomia, Nos. 8 and 9, 1912. Buenos Aires; see also Sartory, A. Champignons parasites de l'homme et des animaux, p. 582. 1922. A species probably belonging to the *A. glaucus* group, described in connection with disease in horses: Colonies forming dusty masses upon corn, at first clear green, later golden green; stalks smooth, erect, from 100μ to 2000μ long and up to 15μ in diameter near the vesicle; vesicles up to 36μ in diameter; sterigmata oval, somewhat longer than the conidial diameter and up to 18 in number, bearing radiating chains of conidia; conidia smooth, green, 4 to 5μ or somewhat larger, swelling to 8μ in germination. No sclerotia or perithecia were found.

Experiments by Quevedo indicated toxicity from consuming material in which this organism had grown, especially for horses and rabbits, but causing little or no danger to sheep and cows. The organism has not since been identified or studied in the light of more recent researches of forage poisoning. Until such repetition is possible the results must now be regarded as questionable.

A. microsporus Böke. The description and figures given by Cattaneo and Oliva in Arch. Lab. Bot. Critt. Garovaglio 5: 123, pl. 6, fig. 9. 1888, have been seen. No earlier or more complete description has been found. The organism was obtained from the human ear and has been listed as *A. fumigatus,* but Wehmer (Monogr. p. 88) notes that the heads are figured as radiate, not calyptrate (Wehmer, Monogr. p. 25), hence suggests this may be *A. glaucus.* See Sartory, A. Champignons parasites de l'homme et des animaux, p. 583. 1922, who quotes only from Saccardo's Latin diagnosis, giving the following: Conidi-

ophores simple, subcontinuous, ending in a clavate vesicle; sterigmata simple, radiating, short fusiform; conidia in chains, spherical, small, green. Culture: No cultural study is recorded. The species may be dropped.

A. pictor R. Blanchard. Sartory (A.), in Champignons parasites de l'homme et des animaux, cites as synonyms *Trichophyton pictor* R. Blanchard, 1895, and *Penicillium pictor* Neveu Lemaire.

No morphological details are given except that macerations of scales from cases of caratés showed mycelium and fruiting branches, some of which resembled Aspergillus, others Penicillium. These cases must be studied more carefully before the identity of such species can be decided. Blanchard described *Trichophyton pictor* (Blanchard, Raphael, pp. 919–922. Parasites végétaux à l'exclusion des bactéries, in Traité de Pathologie Générale, Bouchard 2: 811–932. 1896) from caratés cases, pinta, etc., but confined his description to pathological material, and reports the mold cells adjacent to the conidia as white, 18 to 20 by 2μ, and the conidia as globose or oval up to 8 to 12 by 6 to 8μ, dark yellowish, with dark granules in the yellowish "liquid."

A. polymorphus Moquin-Tandon. Éléments de botanique médicale. 2. éd., p. 469. 1866. This name is cited as given by Pouchet to certain yeasts. It may be dropped.

A. rubens Green (1868). Boston Soc. of Med. Sci. 1868 (19 Nov.). Listed in Wehmer Monogr., p. 127. This specific name has not been noted in the publications seen.

PART II

THE NOMENCLATURE OF THE ASPERGILLI

SECTION I

Group Keys

For convenience in grouping Aspergilli, two shorter keys are presented at this place, one is artificial in being made up mainly without regard to relationship, but is based upon the color of the heads and stalks. The other is an abbreviation of the Synoptical Key presented later, giving the larger groups in the order used in the taxonomic section and followed through the larger Synoptical Key itself. The large majority of specimens or cultures may be located to the group from one of these briefer keys more quickly than from the more extensive discussion or analysis which aims to present the whole genus.

In the dichotomous index system used each symbol ordinarily introduces both members of a contrasted pair of characters. The arrangement of these symbols (either letters or numbers) in the column at the left is in strictly serial order. In the group key (2) the index numbers for each group are practically identical with those used for the same groups for the Synoptical Key (page 221), hence using this briefer key may be a quick method of reaching the desired section of the more elaborate and complicated analysis.

1. Artificial key to groups—primarily based on color

A. Conidial heads green or yellow green.....B
AA. Conidial heads never green.....I
B. Stalks smooth.....C
BB. Stalks pitted (often rough as seen with low magnification).....H
C. Vesicle cylindrical clavate, stalks coarse.....*A. clavatus*, p. 97
CC. Vesicle flask-shaped or globose, not cylindrical clavate.....D
D. Sterigmata 1 series.....E
DD. Sterigmata in 2 series.....F
E. Conidia mostly elliptical and more than 4μ in long axis. Perithecia commonly found, yellow.....*A. glaucus* group, p. 101
EE. Conidia mostly globose 4μ or less in long axis; conidial chains in narrow, solid columns.....*A. fumigatus* group, p. 129
F. Conidial chains in columns.....*A. nidulans*, p. 137
FF. Conidial chains in radiate heads.....G
G. Heads blue green.....*A. sydowi*, p. 147
GG. Heads glaucus, green or yellow-green to buff....*A. versicolor*, p. 142

H. Stalks pitted (often appearing to be rough or asperulate with low magnifications)....*A. flavus-oryzae* group, p. 198
I. Conidial heads never green....J
J. Stalks smooth....K
JJ. Stalks pitted (or apparently rough)....O
K. Conidial heads avellaneous or in brown shades....L
KK. Conidial heads white (or slightly yellowed in age)....N
KKK. Conidial heads brown to black....*A. niger* group, p. 165
KKKK. Conidial heads orange to umber....*A. wentii*, p. 183
L. Conidial heads in columns....M
LL. Conidial heads radiate, stalks colored....*A. ustus*, p. 152
M. Stalks colorless or nearly so....*A. terreus*, p. 150
MM. Stalks yellowed (especially in outer layer)...*A. flavipes* group, p. 155
N. Stalks yellowed, conidial chains in columns ...*A. flavipes* group
NN. Stalks colorless; heads mostly radiate or globose *A. candidus* group, p. 157
O. Stalks pitted or rough.
P. Heads yellow to ochre, radiate....Q
Q. Heads in bright yellow colors....*A. sulphureus*, p. 185
QQ. Heads in ochraceous shades....*A. ochraceus* group, p. 184
PP. Heads orange to umber; conidia rough with tubercles and bars of color....*A. tamarii* and allies, p. 194

2. Key to the larger or more abundant groups in Aspergillus

1. Heads definitely cylindrical clavate, fertile area (vesicle) cylindrical, much longer than the diameter, not globose or elliptical *A. clavatus* group, p. 97
1. Heads not clavate....15
15. Stalk walls smooth not pitted or roughened (superficial crystalline concretions with yellow or red color are common in the *A. glaucus* group). Conidia either rough or smooth....16
15. Stalk walls pitted or rough, sometimes also granular....220
16. Conidial heads some shade of green (including gray shades) (green as a colony color often masked by secreted pigment and crystalline concretions on the hyphae)....17
16. Conidial heads never green....140
17. Sterigmata in 1 series....18
17. Sterigmata in 2 series....100
18. Conidia predominantly more or less elliptical or pyriform, smooth or rough and mostly more than 4μ in long axis; with or without globose, yellow perithecia and ascospores 19 to 53 The *A. glaucus* group, p. 101
18. Conidia predominantly globose, small, rarely over 4μ in long axis; conidial chains aggregated into a column 87 to 93 The *A. fumigatus* group, p. 129
100. Heads with both primary and secondary sterigmata, a few species with simple sterigmata included; mostly green; conidia mostly spinulose....104–115

104. Conidial chains in dense columnar masses; stalk walls more or less colored olivaceous or yellowish brown; perithecia with red ascospores present in part of group.....*A. nidulans* group, p. 137
104. Conidial chains not in columns; stalk walls mostly colorless or nearly so; heads subglobose or with radiating chains; perithecia not found.....124 a and c
124a. Blue-green colonies; orange to red in substratum....*A. sydowi*, p. 147
124c. Glaucous, green, or gray-green, colonies with more or less buff and flesh colored areas; reverse and substratum usually in yellow to red colors, rarely colorless.....*A. versicolor* group, p. 142
140. Conidial heads never green.....141
141. Conidia thin walled, smooth or spinulose, without tubercles or bars of coloring matter, and without pits; not brown, purple-brown or black.....151
141. Conidia roughened with tubercles or bars of color, mostly shades of yellow to purple-brown or to black.....187–214 *A. niger* group, p. 165
141. Conidia more or less irregular, pitted; orange to umber colonies 215 *A. wentii* group, p. 183
151. Conidial walls definitely spinulose.....152
151. Conidial walls smooth or nearly so.....155
152. Stalks uncolored; heads columnar.....*A. terreus* group, p. 150
152. Stalks more or less deeply colored.....153
153. Stalk walls olive gray; conidia coarsely spinulose 3 to 4μ diam.; heads globose, hemispherical or semi-calyptrate, brownish; colonies more or less floccose.....*A. ustus*, p. 152
155. Conidial walls smooth or nearly so.....156
156. Stalk walls yellowed in outer layer.....*A. flavipes* group, p. 155
156. Stalk walls colorless (generalized yellowing occasionally at apex only.....166
166–178. Heads white (or pale yellow when very old)..*A. candidus* group, p. 157
220. Stalk walls pitted sometimes also granular, often reported as rough or echinulate when observed with low magnifications.....221
221. Colonies never green.....223
221. Colonies some shade of green or yellow green.....290
223. Stalk walls in outer layers yellow, often showing concretions or warts; sterigmata in 2 series; heads ochraceous to yellow in varying shades; conidia smooth or only delicately spinulose, with or without sclerotia but perithecia not found..223 to 254 *A. ochraceus* group, p. 184
223. Stalk walls not yellow; conidia rough; heads in shades of brown to umber; sclerotia occasional.....276–279 *A. tamarii* group, p. 194
290. Green or yellow-green colonies (rarely with no green color), without color bars but with walls marked or grooved with winding pits, often giving a falsely echinulate appearance when viewed with low magnification.....292
292. Sterigmata commonly in 1 series, occasionally double. Stalks long; heads yellow with very little green color, large..295 *A. oryzae*, p. 198
292. Sterigmata both simple and double; mostly double in same heads; green color usually abundant.....297 to 307 *A. flavus* series, p. 199

PLATE III

PHOTOGRAPHS OF ASPERGILLUS HEADS TO ONE MAGNIFICATION (× 260)

107. *A. clavatus*, long clavate or elliptical head, the largest of the common species.

111. *A. niger*, black, globose, fairly regular heads, more or less rimose at the periphery, on smooth stalks.

116. *A. wentii*, brown, nearly globose, fairly regular heads, with surface fimbriate, on smooth stalks.

4235I2. *A. tamarii*, brown, irregularly and incompletely globose, fimbriate heads on pitted stalks.

108. *A. flavus*, green, incompletely globose to hemispherical, fimbriate heads, on pitted stalks.

106. *A. candidus*, with large heads globose, fairly regular and periphery more or less loosely fimbriate.

130. *A. effusus*, close to *A. flavus* in appearance, irregularly hemispherical heads, with chains of conidia more or less divergent.

109. *A. amstelodami*, hemispherical heads, with chains of conidia partly diverging, partly tending to form columnar masses.

118. *A. fumigatus*, green to dark green columnar composed of closely massed conidial chains; stalks comparatively very slender.

PLATE IV

PHOTOGRAPHS OF ASPERGILLUS HEADS TO THE SAME MAGNIFICATION (× 260)

4838. *A. terricola* var. *americana*, hemispherical head with loosely diverging chains.

3556. *A. ustus*, hemispherical head, but compact and frequently becoming columnar in age as in *A. terreus*.

2743. *A. versicolor*, incompletely spherical head fairly regular and fairly compact (× 200).

4235I7. *A. sydowi* (from slide), incompletely spherical heads but usually very regular and compact before mounting.

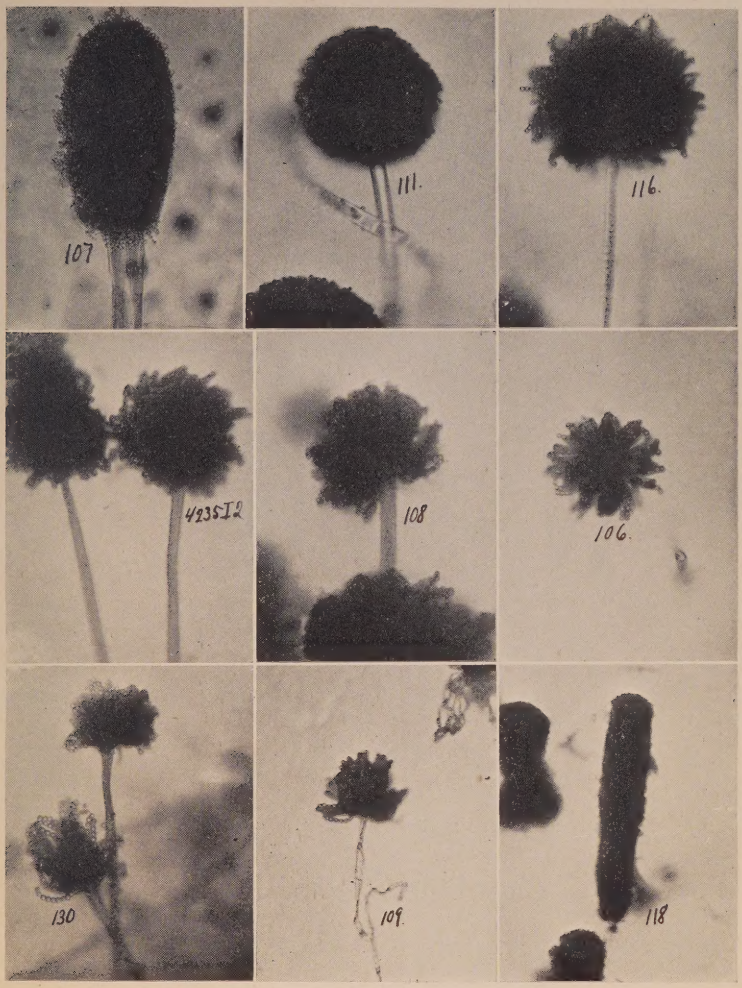
K22. *A. sydowi*, one of the incomplete or abortive heads common in this species (magnification not known).

4666. *A. luchuensis* (from slide), brown globose heads with large vesicle and 1-series of sterigmata.

118. *A. fumigatus* (from slide), a typical figure of this cosmopolitan species.

144. *A. terreus*, brown columnar heads.

114. *A. flavipes* var. ?, white columnar heads.


107
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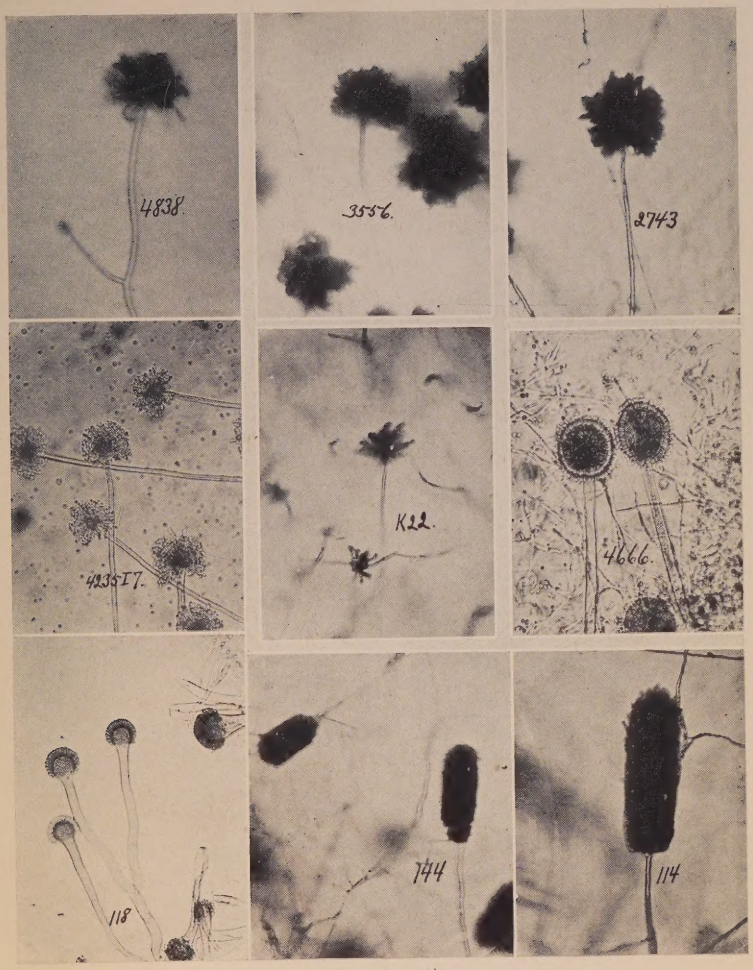
4838.
3556.
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K22.
4666.
118
744
114

SECTION II

The A. Clavatus Group and Associated Species

Heads definitely clavate, fertile area cylindrical clavate, not globose, elliptical or flask-shaped. (Compare p. 101).....The *A. clavatus* group
Sterigmata in 1 series.
Stalks coarse, fairly long, usually several mm. on all media..*A. clavatus* Desm.
Apparently synonymous.....*A. clavellus* Peck
Name attached to specimen apparently near *A. clavatus*
A. westendorpii Sacc. and March.
Stalks very short (dwarf) on Czapek, 1–2 cm. on gelatine and dung cultures
A. giganteus Wehmer 138, 4754.3
Stalks slender.....4202.6 c.
Conidia 2 by 5 to 6μ.....*A. fusco-cinereus* Ellis and Morgan
Sterigmata in 2 series.
Ascosporic, general morphology suggesting *A. clavatus*.....*A. pseudoclavatus*

A. clavatus Desm. Ann. Sci. Nat. Bot. (2) 2: 71, pl. 2, fig. 4. 1834. The original description is "Hyphasmate tenui; floccis sporidiferis albis, simplicibus, sursum incrassatis; sporidiis glaucis, globosis, in capitulum claviforme collectis. Habitat in variis corporibus putrescentibus, in Gallia (v. v.). Cette espèce, trés élégante, forme de petites touffes cendrées ou glauques sur plusieurs substances putréfiées. Elle doit être placée à côté de l'*Aspergillus glaucus*, dont elle se distingue parfaitement par la réunion de ses sporidies en têtes allongées ou claviformes."

Exsiccati: Numerous, but it is scarcely distinguishable from *A. giganteus* except by culture.

Cultures: No. 107 from Kral, in 1909 and many cultures independently isolated by us and by others; fairly common and well-known.

The following English diagnosis from culture is a composite from the study of many strains (compare fig. 10 and plate II). Colonies in Czapek's solution agar with cane sugar, gray green to dark green, densely matted, heavy, rapidly growing and spreading; reverse and agar not colored in young colonies, more or less brownish when old; conidiophores with walls smooth, colorless, rather thin, up to 1 to several millimeters in length, commonly 15 to 20μ in diameter, gradually enlarged at apex into a clavate vesicle which is fertile over an area up to 150μ long by 20 to 25μ in longest diameter; sterigmata 7 to 10 by 2 to

3μ, in a single series densely covering the fertile area, bearing long chains which frequently adhere more or less into masses; conidia elliptical, green, 2.5 to 3 by 3.4 to 4.5μ, smooth, thin-walled, swelling to 5μ diameter and germinating by a single tube (frequently 350μ long by 2 to 2.5μ in diameter in 18 hours). No perithecia found.

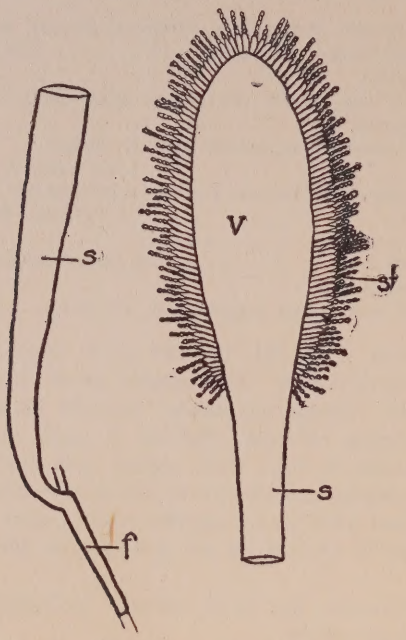

FIG. 10. A. CLAVATUS; OUTLINES OF OPTICAL SECTION OF HEAD WITH CAMERA LUCIDA

Details diagrammatic; *s*, stalk; *v*, clavate vesicle; *st*, sterigmata in a single closely packed series bearing chains of conidia.

Colonies of this species grow better at 20° than at 37°C., but grow well under both conditions, grow poorly in media with 10 per cent NaCl added, liquefy gelatine both with and without sugar, but produce small colonies; digest milk slowly without normal spore production, produce very short conidiophores with green fruit in medium with 40 per cent cane sugar, very long conidiophores with slow development of green color in medium containing 6 per cent beef extract.

A. clavellus Peck and *A. westendorpii* of Saccardo and Marchal do not appear to be separable.

A. clavellus Pk. N. Y. St. Mus. Nat. Hist. Rept. 34: 49, pl. 2, fig. 1–5. 1881. Syn. for *A. clavatus* Desm. Described from cooked squash, in New York State, but the author presents no observations to warrant separating *A. clavellus* from *A. clavatus* of Desmaziéres; the conidia were elliptical 4 to 5μ in long axis.

Culture: None.

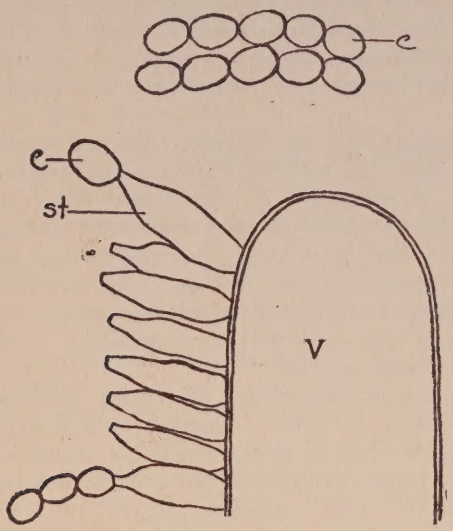

FIG. 11. A. CLAVATUS; A FEW STERIGMATA, *st.*, MUCH ENLARGED WITH THE ELLIPTICAL CONIDIA, *c.*

A. westendorpii Sacc. and March. Rev. Mycol. 7: 149. 1885. Complete reference: "In fimo vaccino. Leg. Westendorp." Lindau in Deutsch. Krypt. Fl. Pilze 8: 152, adds that the specimens are apparently *A. clavatus* or a variety of that species.

Culture: None; not seen by us; to be dropped.

A. giganteus Wehmer. Centralb. f. Bakt., etc. 2 Abt., 18, No. 13/15: 385. 1907.

Colonies in Czapek's solution agar bluish green; stalks short up to 500 to 700μ long by up to 60μ diam., vesicles up to 70 to 80μ in diameter and 150 to 200μ long; sterigmata in one series closely covering the vesicle

7 to 8.5 by 2 to 3μ. Conidia about 3 by 4μ, elliptical, smooth. When grown in horse manure, and various other media containing organic nitrogen, stalks become 1 to 2 cm. in length with enormous masses of conidia.

Culture: By Wehmer; by Thom No. 138 from Westerdijk, from Linder 4754.3.

Wehmer reports a comparative study of *A. giganteus* and *A. clavatus*, upon which he justifies the separation of the species. In our cultural studies, *A. clavatus* produces coarse stalks upon practically all media, while *A. giganteus* depends upon special nutrients to produce its long stalks and large heads. Under such conditions, the unique characters of this species become very conspicuous thus justifying Wehmer's description.

A. fusco-cinereus Ellis and Morgan. MS. name attached to Morgan's specimen No. 674, with the following description in Ellis' longhand on the inside of the packet: "Fertile hyphae 200 to 250 by 5 to 6μ, brownish and septate below, hyaline and continuous above and inflated into a subcylindrical head 40 by 15μ clothed with a layer of narrow, elliptical hyaline conidia (?) 5 to 6 by 2μ on very short sterigmata."

Our examination confirms the above description, which places this form tentatively near *A. clavatus* morphologically. No other specimen of this form has been reported.

A. pseudo-clavatus Purjewitch. In[1] Schrift. Naturforsch. Gesell. Kiev. 16, 2, 309, pl. 12. 1900. See Saccardo Sylloge Fung. 16: 1028.

Conidial areas white to intensely green, later gray green. Stalks 3 to 5 mm. in length, 25 to 35μ in diameter at base, increasing to the vesicle which is 60 to 70μ in diameter and 260 to 300μ long; sterigmata closely packed in 2 series, primary 8 to 9μ, secondary delicate 2 to 5μ in length; conidia oval, grayish green, 3.5 to 4 by 2.5 to 3μ; perithecia develop only under special conditions, 60 to 70μ in diameter, with asci 7 to 8μ, and 6 to 7 in the perithecium. Ascospores colorless, oval. Separable from *A. clavatus* by its double sterigmata and perithecium production.

Cultures: Purjewitch; not seen by us.

[1] This Russian article has been commonly cited under the German translation of the name of the journal; this practice is continued. The data given in the description are taken from the original.

SECTION III

The A. Glaucus Group

Heads not clavate.
Stalk walls smooth, not pitted or roughened, with conidia either rough or smooth. Superficial granules or crystalline concretions are often found but no pits or roughenings of the stalk wall occur (compare p. 183)
Conidial heads green or greenish, gray or almost white forms occur but with close morphological affinities to green forms (compare p. 149 for heads of other colors)
Sterigmata in 1 series (compare p. 137)
Conidia mostly more than 5μ in long axis (compare p. 124 and p. 129).....The *A. glaucus* group

A. glaucus group: species known in culture or probably identifiable.

Ascospores large, more than 8μ in long axis.
A. herbariorum series *major* (Mangin) Thom and Church.
A. herbariorum series *major* (Mangin) var. *violaceum* (Mangin) Thom and Church.
A. echinulatus (Delacroix) Thom and Church.
A. medius Meissner.
A. disjunctus Bainier and Sartory.
A. repandus Bainier and Sartory.
A. mencieri Sartory and Flament.
Ascospores intermediate in size, 6μ or more in long axis.
A. herbariorum series *minor* (Mangin) Thom and Church.
A. umbrosus Bainier and Sartory.
Ascospores less than 6μ in long axis.
A. chevalieri (Mangin) Thom and Church.
A. sejunctus Bainier and Sartory.
A. ruber (Spieckermann and Bremer) Thom and Church.
A. scheelei Bainier and Sartory.
A. scheelei var. *B.* Bainier and Sartory.
A. amstelodami (Mangin) Thom and Church.
A. repens (Corda) Saccardo.

A. glaucus group: synonyms, doubtfully identifiable and inadequately described species.

E. amstelodami Mangin.
A. argentinus Speg.
A. brunneofuscus Sée.
A. caesiellus Saito.
A. carneolus Sacc.
E. chevalieri Mangin.
E. chilense Montagne.
A. cinerescens Bainier and Sartory.

E. coriorum Wallroth.
A. delacroixii Sartory and Sydow.
E. desmazieri Castagne.
E. diplocystis B. & Br.
E. echinulatum Delacroix.
E. epixylon Kunze and Sch.
A. ferrugineus Fries.
A. ferrugineus Link.
E. fructigenum Link.
A. giganto-sulphureus Saito.
A. glaucus Link.
A. glaucus var. *albida* Speg.
E. *glaucus* var. *minimus* Hanzawa.
A. glaucus var. *oblongisporus* E. & W.
A. glaucus var. *olivascens* Sacc.
A. glaucus var. *repens* Corda.
A. glaucus var. *subolivaceus* Ferraris.
A. godfrini Sartory and Roederer.
E. lateritium Montagne.
A. macrosporus Bonorden.
A. mencieri Sartory and Flament.
A. mollis Bainier & Sartory.
A. nanus Oudemans.
A. ochraceo-ruber Saccardo.
A. olivascens Saccardo.
A. olivaceus Delacroix.
A. olivaceus Saccardo.
A. penicillatus Greville.
A. penicillatus Link.
A. rufescens Berlese.
E. sacchari Spegazzini.
A. sartoryi Sydow.
A. spiralis Grove.
A. stercoreus Sacc.
E. verruculosum Vuillemin.
A. virens Link.

THE A. GLAUCUS GROUP

Summary of characters

Heads green; stalks smooth (not rough or pitted) septate (articulate); sterigmata 1 series; conidia predominantly elliptical more than 5μ in long axis (with a few exceptions), mostly with roughly pitted wall, rarely smooth; most races or species producing sessile globose, cleistocarpic yellow to orange perithecia with membranaceous walls, borne above the surface of the substrata and producing disk-shaped ascospores colorless or nearly so.

Range of variation in characters

Conidial heads some shade of green, or gray-green during the growing period; color of heads in the colony frequently masked by the development of yellow to red granular incrustations of the aerial sterile hyphae and by red or yellow pigment in the substratum and frequently by the yellow to orange color of the perithecia when abundantly produced and which dominate the colony color reported. Stalks septate (articulate), with walls smooth, not pitted or roughened but often bearing colored concretions, either produced as very short branches of aerial mycelium or long and arising directly from the substratum in various strains and under various conditions of growth; vesicles varying from only slight apical dilations of the stalk to globose; sterigmata in one series, usually fairly large, and often loosely clustered and in many strains occasionally

proliferating to become short secondary stalks with small heads. Conidia elliptical to oval or globose in some strains and usually more than 5μ in long axis (except *A. repens*, *A. amstelodami*, *A. penicilloides*) in chains usually showing a connective, mostly thick-walled, rough or echinulate (due to a broad open type of pitting), in a few species smooth, showing more or less green color in mounts.

The members of the *A. glaucus* group are mostly tolerant to fairly high osmotic concentrations in nutrient substrata; they are, therefore,

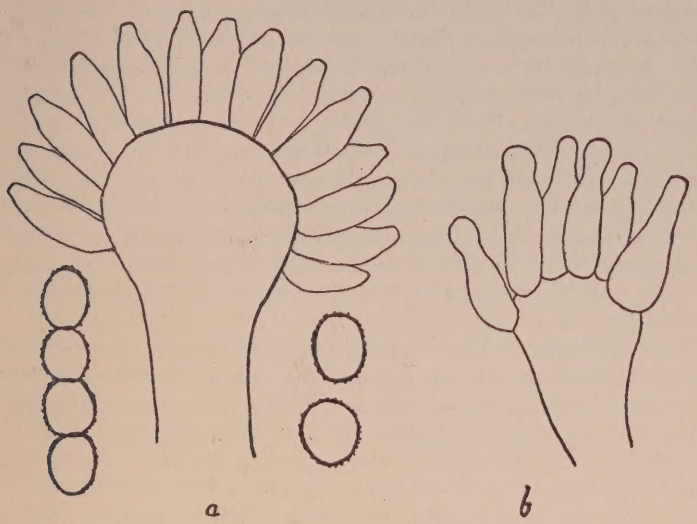

FIG. 12. TWO HEADS BELONGING TO THE A. GLAUCUS GROUP

a, large with radiating sterigmata rather closely packed; *b*, small with a few loosely arranged sterigmata on the apex.

abundant on dried or partly dried plant products, hay, fodder, forage of all kinds, musty cereal products slightly exceeding the lowest limit of moisture for growth, stale bread, salted meats and fish, nuts in the shell, preserves, jellies, jams, soft sugar products, leather goods, fibers, and similar situations in which they cause a very large percentage of all the moldiness encountered. Members of the *A. glaucus* group are found upon substrata in every stage from moist to very dry conditions and respond by great differences in habit of growth. Upon dilute substrata some forms produce abundant short stalks and conidia-bearing heads

with or without sterile aerial mycelium and with or without perithecia. In some of them, spore formation is almost suppressed in dilute areas, but appears abundantly in more concentrated areas as coarse long stalks, large heads of conidia and abundant perithecia borne upon scanty networks of hyphae on the walls of the culture tube several millimeters away from moist areas of nutrient media. Between such extremes, many intermediate stages represent the responses of the variant strains to the concentration of substrata.

In attempting to classify this aggregation of organisms, the two extremes are represented by Mangin (loc. cit.), who groups all but two or three forms into three series under Eurotium, as *E. herbariorum* series *major* Mangin, series *minor* Mangin and *E. repens* De Bary, and in contrast by Bainier and Sartory, whose description of separate species will be found in the paragraphs which follow. For the purposes of routine identification, Mangin's rough grouping is very useful. When a study of particular organisms becomes significant, Bainier and Sartory have furnished an equally useful basis for the placing of some, though by no means all of the variant strains which differ in habit and in biochemical activity, accompanied by more or less definite responses in morphology.

The following forms have been reported from cases of disease, hence as presumptively pathogenic: *A. mencieri*, from a pulmonary mycosis, *A. fontoynonti* and *A. tokelau* from skin lesions, *A. repens* from infected ears, and *A. herbariorum* questionably identified in two cases.

A. glaucus Link, Obs. p. 16. 1809. See Key Nos. 19 to 53. Link's combination appears to be the genus Aspergillus attributed to Micheli and *Monilia glauca* of Persoon's Synopsis, p. 691. From the time of Link's Observationes 1809, to the work of De Bary and Woronin, *A. glaucus* appears to be used for the green conidial stages, and the name Eurotium for the perithecial forms of Aspergilli found upon badly dried specimens in phanerogamic herbaria. Mangin (Ann. Sci. Nat. Bot. 9 sér., 10, p. 360. 1909) is probably correct in saying that there is no possible means of identifying the name *A. glaucus* with one only of the several forms occurring on such specimens, since such identity can only be safely based upon the study of ripe ascospores. To one who has examined many such moldy specimens, Greville's figure of *A. penicillatus* becomes instantly recognizable as belonging in the series and equally unrecognizable except as a member of the series. The name *A. glaucus* may then be discarded as a designation of a particular strain and broadened to cover a series concept which will include all of the green

Aspergilli having certain well marked common characters, which clearly indicate close relationship.

In this sense, this group of forms is probably the most abundant and the most commonly known of all the Aspergilli, on account of their frequency in the herbarium and upon commonly used food products, and their abundance and massive production of mycelium and yellow to orange perithecia upon hay, fodder and other forage which is just moist enough frequently from absorption of moisture from humid atmosphere to permit these forms to grow with few competing organisms.

Eurotium Link, Obs. p. 31. 1809. See *A. glaucus* group for discussion. For its historic interest Link's generic description may be repeated here. It was undoubtedly based upon yellow perithecia of the *A. glaucus* group as found upon badly dried specimens in phanerogamic herbaria. "Sporangium globosum, sessile cinctum thallo floccoso. Peridium membranaceum, sporidia coacervata." "Praecedenti generi valde affine et sporangium idem. Stipites vero nulli, horum loco thallus tenuissime floccosus, e floccis fuscentibus septatis sporangia cingit Peridium tenue aqua adfusa rumpitur et sporidia majuscula globosa effundit. Est mucor herbariorum Pers. Cujus iconem V, fig. 44."

No attempt to cite all species of Eurotium has been made in this paper. The species of Eurotium cited are those which clearly suggest the probability that they belong with the Aspergilli. Some species described under the name are manifestly members of other genera.

Such species of Eurotium as are given in the following list are excluded from Aspergillus by the descriptions given for them:

E. caulineola Fries, Sys. Myc. 3: 334. 1832.
E. creatinum Nyl., An. Myc. in Fl. Fenn, p. 126.
E. diplocystis B. and Br., Jour. Linn. Soc. [London] Bot. 14: 137. Tab. 10. fig. 55–56. 1875.
E. fuscum Preuss, Linnaea 25: 78. 1852.
E. nebulosum Fries, Sys. Myc. 3: 334. 1832.
E. pulcherrimum Winter. Rabh. Krypt. Fl. 2 Aufl. 1. Abt. 2: 60. 1887.
E. pyrobolus Fries, Sys. Myc. 3: 333. 1832.
E. rosarum Grev. Scottish Flora 3: 164. 1825.
E. semi-immersum El. Marchal, C. R. Soc. Roy. Bot. Belg. 33: 128, Pl. II, fig. 3. 1895.

In analysing this group to identify particular forms with existing literature, the ascospore is accepted as the principal basis of separation.

Ascospores with furrow and crests, large

A. herbariorum (Wiggers);[1] Syn. *Eurotium herbariorum* Link, Obs. p. 31, Taf. 2, fig. 44. 1809. See also Mangin, L., Ann. Sci. Nat. Bot. 9 Série, 10, p. 365. 1909. According to Mangin: Mycelium white, to yellow, yellow-orange or red orange on sugared carrot; with development of conidia ashy green, glaucous, or olive, in age greenish-black; all forms produce in the mycelium a pigment, yellow-orange changing to violet with alkali; perithecia abundant, sulfur yellow; conidia mostly oval or elliptical from 6.6 to 7.5 by 9.4 to 11μ, occasionally globose 6.6 to 8.4μ in diameter. Ascospores with furrow and crests. Two series were based on the size of the ascospores: Series *major* Mangin about 9.6 by 6.6μ and series *minor* Mangin about 7.5 by 5.8μ.

Mangin in contrast to Bainier and Sartory groups a large number of strains together under *E. herbariorum* series *major*. All of these forms have large ascospores with furrow and crests, while the members of his series *minor* have smaller ascospores. Following Mangin, *A. medius*, *A. echinulatus*, *E. verruculosum*, *A. disjunctus*, *A. repandus*, and *A. mencieri*, would belong to series *major*. Similarly, *A. umbrosus*, *A. mutabilis*, *A. mollis*, and associated strains having ascospores larger than 6.6μ in long axis would belong to series *minor*.

Ascospores with furrow and crests more than 7.5μ in longest diameter

A. herbariorum series **major** (Mangin); syn. *E. herbariorum* series *major* Mangin. Ann. Sci. Nat. Bot. 9 Sér., 10: 365. 1909.

A whole series of nearly related strains with large ascospores showing an equatorial furrow and bands or crests has been covered in Mangin's proposed "series major." Bainier and Sartory in describing certain members of this series as species have emphasized biochemical differences conspicuous in a selected group of strains. Whether positive identification of these species can be accomplished except by accurate quantitative study is doubtful.

A. herbariorum series **major** var. **violaceus** (Mangin); syn. *E. herbariorum* series *major* var. *violaceum*. Ann. Sci. Nat. Bot. 9 Série, 10, p. 369. 1909.

Mycelium white becoming rapidly rich violet or orange then violet

[1] Fischer in Engler and Prantl, Die Natürlichen Pflanzenfamilien (1 Teil, Abt. 1, p. 301. 1896) discards Sterigmatocystis and Eurotium and adheres to Aspergillus as the proper designation for the whole group. Just who used the combination *A. herbariorum* first has not been settled; it certainly occurs this way in Fischer's discussion.

black; conidia very large, globose or oval, prominently verrucose and attaining a size of 15 to 18μ; ascospores large, with distinct furrow and ridges, 9.4 by 6.6μ.

E. lateritium Montagne, Centurie VI, no. 45, in Ann. Sci. Nat. Bot. 3 Sér., 11, p. 54. 1849, and Sylloge, p. 257. Syn. *E. herbariorum* series *major* Mangin. Colonies at first yellow then orange, finally brick red; perithecia 120 to 250μ diameter, very abundantly produced; asci about 20μ in diameter, 8 spored; ascospores hyaline, 7 to 10μ in diameter, with frill?

This species was found closely associated with *Aspergillus herbariorum* on moldy bread and described fully enough to fix the group to which it belonged.

A. echinulatus (Delacr.); Syn. *E. echinulatum* Delacr. (Delacroix, G. Bul. Soc. Myc. France 9: 266. Pl. XIV, fig. III. 1893). Syn. *E. verruculosum* Vuill. Perithecia sulphureus, about 140μ in diameter; asci globose 15 to 20μ in diameter, 8 spored, with sporidia about 11 by 7μ, with shallow furrow, discoid, hyaline, slightly echinulate and bearing echinulations (shown in the figure) upon the faces as well as the edges of the valves. Reported as the ascosporic stage of *A. brunneus*, presumably of Delacroix. There is substantial identity between the ascospores of *E. echinulatum* and those described by Vuillemin for *E. verruculosum*. This morphology is repeated in our no. 4481, with measurements of Delacroix's description, but accepted by Vuillemin for his species; the conidial form is described as follows:

A. brunneus Delacroix. Bul. Soc. Myc. France 9: 185. Plate XI. fig. III. 1893. Cited by Delacroix as the conidial stage of *E. echinulatum* Delacroix. Colonies at first green then dark brown, floccose; conidiophores up to 15μ in diameter, enlarging into a subglobose vesicle up to 60μ in diameter, bearing sterigmata about 12 to 18 by 5 to 7μ; vesicles, sterigmata and conidia fuscous; conidia globose, echinulate, about 15μ diam.

Culture: By Delacroix.

A. brunneus is cited by Thom and Currie (Jour. Agr. Res. 7: 12. 1916) and incorrectly attributed to Bainier.

E. verruculosum Vuillemin. Bul. Soc. Myc. France 34, no. 1/2, p. 83. 1918. Syn. for *E. echinulatum* Delacr.

Colonies closely felted floccose, with dull green heads intermingled with hyphae crusted with red granules; conidiophores up to 22μ in diameter; vesicle oblong 20 to 40μ; sterigmata 13 to 17.5 by 6.6 to 7.6μ; conidia subglobose 10 by 8 to 9μ or about 9.5μ rough. Perithecia scant-

ily produced on the driest edges of the culture medium and on the glass; ascospores 9 to 10 by 6μ with furrow and uneven wavy crests attached to the valves of the ascospores and markings continued over the faces of the valves.

Culture: By Vuillemin, but type lost.

Our no. 4481 isolated from figs in California by Miss Phillips has been verified by Vuillemin. However careful examination of the description and figures of *E. echinulatum* Delacr. (1893) indicates the identity of Vuillemin's form as a redescription of Delacroix's species.

A. medius Meissner. Bot. Ztg. (2) 55: 337–344, 353–357. Meissner described a member of the *A. glaucus* series apparently intermediate between *A. herbariorum* ser. *major* and ser. *minor* of Mangin; with felted woven mycelium, becoming colored in age by encrustment of hyphal walls with coloring substance orange-yellow in acid solution and red to violet in ammoniacal solution; stalks colored as the vegetative hyphae, 5 to 8.3μ in diameter (length not given); vesicles 12 to 35μ in diameter; sterigmata covering the upper $\frac{1}{2}$ to $\frac{3}{4}$ of the vesicle, up to 13 by 6μ; conidia at first smooth, then rough, 7 to 12 by 6 to 10μ; perithecia 83 to 125μ in diameter; asci 20 to 25μ in diameter; ascospores viewed on the flat side about 12 by 8μ, on edge about 12 by 4 to 6μ, with frills separated by a scarcely defined furrow, colorless, smooth.

A culture (137) received from Westerdijk in 1909 and lost by us, was apparently identical with one obtained from Raistrick (4724.45) in 1924. In these cultures few perithecia were found; ascospores up to 10 by 6μ were found with frills and furrow; conidia roughened and up to 10 to 12 by 4 to 6μ were borne in close aggregates of conidial chains forming nearly a solid column from the apex of the vesicle. The colonies grow slowly and become intensely red-brown almost black in age. Possibly this strain is the type of Meissner's species, but records are lacking. Cultures grew better in the ice-refrigeration at 10° to 14°C. than at higher temperatures.

A. disjunctus Bainier and Sartory. Bul. Soc. Myc. France 27: 346–368, pl. X–XI. 1911. A member of the section of the *A. glaucus* group, with red pigment formation. Colonies with conidial areas yellow-green; stalks 280 to 840 by 14 to 16μ; vesicles 42 to 56μ from hemispherical to globose; sterigmata forming with conidial chains a fairly compact mass on the upper hemisphere of the vesicle; perithecia about 224μ in diameter, produced within a few days; ascospores 11.2 by 5.6μ with furrow and crests; conidia smooth or echinulate, 14 to 17μ in long axis, pyriform or "top-shaped." Culture: By Bainier and

Sartory, with biochemical studies. This organism has not been identified by us, but would probably be found in a continued survey of the varieties of this great group.

A. godfrini Sartory and Roederer. Études biologique et morphologique d'un champignon thermophile du genre "Aspergillus" (*Asp. godfrini* n. sp.). In Assn. Française pour l'Avancement des Sciences, 42nd Session, Tunis, 1913, p. 601–603. 1914. Colonies yellowish brown from ripening conidia; reverse uncolored; without perithecia; stalks 300 to 780 by 12 to 18μ; vesicle globose 40 to 50μ diameter, bearing sterigmata on the upper part only and chains of conidia in solid columns; conidia elliptical to globose, yellow-brown, smooth, varying in size up to 8 to 12μ in long axis, or occasionally larger. Culture: By Sartory and Roederer; not seen by us. See Key 23h and 267c.

The describers suggest a resemblance to members of the *A. glaucus* group described by them, but describe it in terms which would place it near the *A. tamarii* group in the color of the conidia. Its real affinities are not clearly brought out by the description, since organisms belonging with *A. tamarii* as far as seen have stalks with walls pitted. The describers found its optimum growth temperature between 35° and 36°C. with growth continuing at 44° or 45°C., but stopped at 47°C. Cultures coagulated and peptonized milk, liquefied gelatin, but were not pathogenic to laboratory animals (rabbits and guinea pigs).

A. repandus Bainier and Sartory. Bul. Soc. Myc. France 27: 463, pl. XVIII. 1911.

A vigorously growing, ascosporic member of the *A. glaucus* group, at first green from early development of conidia, becoming later violaceous; perithecia very abundant, orange yellow, up to 140μ diameter; asci about 17μ diameter; ascospores 11 by 6μ, with furrow, but no frill or crest; stalks long with globose vesicles and radiate heads; conidia rough, large.

Cultures by Bainier and Sartory showed an optimum growth temperature between 23° and 26°C., red pigment in the substratum.

A. mencieri Sartory-Flament. (Sartory, A., and Flament, L. In Sartory, A. Champignons parasites de l'homme et des animaux, 8e fasc., p. 578. 1922; see also Sartory, A., and Flament, L. Étude morphologique et biologique d'un *Aspergillus* nouveau isolé d'expectorations d'un malade suspect de tuberculose pulmonaire. Compt. Rend. Soc. Biol. [Paris] 83: 1114–1115. 1920; and Sartory, A., and Bailly, A. Les mycoses pulmonaires et leur parasites, Paris, pp. 180–181. 1923.)

As described: Stalks uncolored at first, then greenish, and in age brownish, abundant 220 to 740 by 14 to 16μ; vesicles abruptly globose 30 to 50μ in diam., or in form of an inverted truncated cone; sterigmata clustered on the apex of the vesicle; conidia with prominent connective, varying, mostly 9 to 12μ in long axis, green to dark gray, smooth and echinulate forms in the same chain, with occasional small conidia and frequent monstrosities; perithecia yellow, 200 to 220μ; asci 15 to 20μ; 8 spored; ascospores 10 by 4.7μ, with furrow and crests.

Culture: Sartory-Flament, Sartory-Bailly: It grows well upon laboratory media, liquefies gelatine, coagulates milk, digests casein, but does not act upon starch, rice, or egg albumen. It ferments glucose, but not maltose, lactose, galactose, or levulose. It is not pathogenic to laboratory animals.

Ascospores with furrow and commonly also ridges or crests, 6.6 to 7.5μ in long axis

A. herbariorum ser. **minor** (Mangin); syn. *E. herbariorum* series *minor* Mangin. Ann. Sci. Nat. Bot. 9 Sér., 10, p. 365. 1909.

In this series Mangin brought together a group of strains of the *A. glaucus* group producing ascospores with furrow and crests or ridges and with long axis from 6.6 to 7.5μ. These limitations would probably include *A. umbrosus*, *A. mutabilis* and *A. mollis* as described by Bainier and Sartory, together with many other strains differing more or less widely in appearance and measurements, as well as in biochemical activities.

A. umbrosus Bainier and Sartory. Bul. Soc. Myc. France 28: 267–268, pl. XII. 1912.

Conidial phase fairly typical of the *A. glaucus* group; colonies dark green; mycelium colorless to yellow, stalks variable in measurement, mostly arising (as figured) as branches of aerial hyphae; vesicles oval to globose; sterigmata not specifically described, but figured as typical of the group and radiating from the upper three-fourths of the vesicle; conidia oval 5 to 9μ in long axis, figured as smooth; perithecia abundantly produced, golden yellow to brick red in age, up to 168μ in diam.; asci about 16.8μ diameter; ascospores 8 by 5.6μ with a definite furrow.

Cultures and biochemical study by Bainier and Sartory showed a deep red pigment chemically identical with that of *A. repandus*, but difference in the size of the ascospores.

A. mutabilis Bainier and Sartory. Bul. Soc. Myc. France 27: 458, pl. XVII. 1911.

Colonies described as at first yellow, then ochraceous, through orange shades to green; with perithecia occasional, 200μ in diameter; asci 16μ and ascospores 8.4 by 5.6μ, with a definite furrow; conidiophores long; vesicle claviform, with radiating chains of conidia; sterigmata scarcely distinguishable from conidia in size or shape, readily separating from the vesicle; conidia oval, smooth, varying from 2 to several times longer than broad, measurements not given.

Culture and biochemical study by Bainier and Sartory showed a temperature optimum of 23° to 24°C., growth on all common media, no liquefaction of gelatine, no alcohol, positive for invertase and with a red pigment insoluble in water, but soluble in a very dilute solution of potassium or sodium hydroxide.

A. mollis Bainier and Sartory. Bul. Soc. Myc. France 27: 453, pl. XVI. 1911. Not *A. mollis* Berkeley, p. 211.

An ascigerous member of the *A. glaucus* group, with perithecia 140 to 168μ in diameter; asci 16.8μ; and ascospores 8.4 by 5.6μ, with furrow only; with conidial areas green to blue-green; some red pigment in the mycelium; head with radiating chains; conidia oval-elongated to spherical, up to 8.5 to 14μ in long axis, figured as smooth.

Culture: Bainier and Sartory: not identified by us.

Prior use of the name invalidates the application of *A. mollis* to this form. If the organism of Bainier and Sartory is found distinct enough to warrant the maintenance of a separate species some other name must be found.

Ascospores with furrow and crests, 4.7 by 3.3μ

A. chevalieri (Mangin), syn. *E. chevalieri* Mangin (pp. 361–362, in Mangin, L. Qu'est-ce que l'Aspergillus glaucus? In Ann. Sci. Nat. Bot. 9 Sér., 10, pp. 303–371. 1909.)

Colonies more or less floccose, becoming green, glaucous or olive with the development of conidia; conidia ovoid to globose, verrucose, averaging 5.6 to 7.5μ in long axis; perithecia abundantly produced at 25°C., rarely at lower temperatures; differing from other members of the *A. glaucus* group in producing ascospores 4.7 by 3.3μ, with distinct furrow and ridges.

Culture: Mangin. Apparently satisfied by 4684P.

Ascospores without prominent crests, frills or ridges, with or without furrow, mostly less than 6μ in long axis

A. sejunctus Bainier and Sartory. Bul. Soc. Myc. France 27: 346–368, pl. X–XI. 1911.

An ascosporic form of the *A. glaucus* group, producing yellow perithecia 156μ in diameter, with asci 11.2μ, and ascospores 5.6 by 4.2μ with furrow only; colonies richly growing, with aerial masses up to 1 cm. high, at first white, then clear green, finally reddish (rougâtre); stalks up to 330 to 642 by 14μ, borne as perpendicular branches of aerial hyphae; vesicle 24 to 42μ, globose, completely covered with long, large sterigmata, producing a radiate or, at least, hemispherical head; conidia smooth or rough, mostly spherical 2.8 to 5.6μ, very variable.

Culture and biochemical study: Bainier and Sartory.

A. ruber (Spieckermann and Bremer). Syn. *E. rubrum* Spieckermann and Bremer, Descr. p. 92 in article, entitled "Untersuchungen über die Veränderungen von Futter-und Nahrungsmitteln durch Mikroorganismen. I. Untersuchungen über die Veränderungen fettreicher Futtermittel beim Schimmeln." Landw. Jahrb. 31: 81–128. 1902.

Colonies more or less floccose, at first white then bluish green with developing conidia, then through yellow to rusty red throughout and intense red in the substratum; stalks arising either from submerged hyphae or from aerial hyphae from 120μ long when forking from aerial hyphae, to 300 to 500 or even 800μ by 8 to 16μ in diameter; vesicle clavate to subglobose, 24 to 30μ in diameter; sterigmata radiating, 4 to 12μ, mostly 6 to 10μ by 2 to 4μ; conidia ovate to globose 5 to 8μ in long axis, rough, pale green (blue green in mass); perithecia globose, yellow then rusty brown, 80 to 120μ in diameter; asci 10 to 12μ in diameter; ascospores 6 by 4 to 5μ with shallow smooth furrow, no frill or ridges.

Culture and biochemical study: Spieckermann and Bremer.

A member of the *A. glaucus* group, in the section with *A. repens* and representing the intense red (alkaline) phase of this polymorphous and polychromatic group.

A. scheelei Bainier and Sartory. Étude biologique et morphologique de certain Aspergillus (suite). Bul. Soc. Myc. France 28: 257–262, pl. X. 1912.

Colonies predominantly green, yellowed somewhat by the development of perithecia and sometimes becoming brown in age, and producing yellow pigment in the substratum; stalks figured (not described) as short branches of aerial hyphae with compact heads forming nearly hemispherical spore masses or splitting into columns in age; sterigmata figured in one series closely packed, rather short, elliptical; conidia oval becoming fairly uniformly globose, with a trace of roughening shown in the figures, 4.2 to 8.4μ in long axis; perithecia very abundant, up to

126μ in diameter; asci 11.6μ in diameter; ascospores 5.6 by 4.5μ, with more or less definite furrow.

Culture and biochemical discussion: Bainier and Sartory; not definitely identified by us in culture.

A. scheelei var. *B.* Bainier and Sartory. Bul. Soc. Myc. France 28: 262–267. 1912. Incorrectly headed *Aspergillus B.* variété *Scheelei.*

Colonies green, very slowly brown, floccose; stalks very numerous, up to 448 by 14μ, with vesicles 28 to 42μ; heads more nearly spherical than *A. scheelei* as figured; sterigmata crowded 4 to 5 by 1 to 2μ; conidia regularly spherical, mostly 4 to 6μ (occasionally 7μ) in diameter, only showing elliptical conidia in old culture (?CT.); perithecia up to 98μ diameter, very abundant, golden yellow with wall thin, easily breaking down; asci 11μ diameter; ascospores 5 by 4μ, with scarcely a trace of furrow.

Culture and biochemical study: Bainier and Sartory.

The form described was close to if not identical with *A. amstelodami* of Mangin. Several cultures approximating this description have been examined.

A. amstelodami (Mangin). *E. amstelodami* Mangin (Mangin, L. Qu'est-ce que l'Aspergillus glaucus? Ann. Sci. Nat. Bot. 9 Sér., 10, p. 360–361. 1909. Compare Plate II, 109). Colonies floccose, tardily green, glaucous or olive green; conidia green, globose, finely echinulate, mostly 2.8 to 4.7μ, or more or less elliptical and occasionally somewhat larger, varying with the temperature, becoming sometimes 5 to 6μ or 5.6 by 7.5μ at 10°C. Perithecia numerous, yellow, deeply surrounded by floccose mycelium; ascospores with furrow only, hyaline, smooth 4.7 by 3.7μ.

Culture: Mangin.

Fairly represented by our 109, which was received from Amsterdam. Occasionally a culture is obtained with ascospores regularly and fairly persistently as small as those described for *A. amstelodami.* In many of the closely related strains, however, occasional ascospores of these particular measurements are found among many somewhat larger, while the definiteness of the furrow, which is the chief character separating these forms from *A. repens,* varies considerably with the culture.

A. glaucus var. *repens* Corda, Icon. 5: 53. 1842. Syn. *A. repens* De Bary q.v.

A. repens (Corda) Saccardo, Syll. 4: 64; syn. *E. repens* (Corda) De Bary and Woronin, in Beiträge zur Morphologie und Physiologie der Pilze, p. 379. 1866. Syn. *A. glaucus* var. *repens* Corda, Icon.

5: 53, Taf. II, fig. 24. 1842. See also Dale, E. Ann. Mycologici 7: 215–225, pls. II and III. 1909, and Mangin, L. Ann. Sci. Nat. Bot. Sér. 9, 10, p. 363. 1909. Abstract of diagnosis from De Bary and Woronin, Dale, and Mangin. Stalks and heads varying greatly in measurements; conidia globose or oval, varying from 4 to 8.5μ in long axis, verruculose; perithecia yellow, small; ascospores 4.7 by 3.7μ, colorless, with edge thick, without ridges or frill and without definite furrow.

Culture: De Bary and Woronin, Dale, Mangin, and many others.

A. repens in this sense in our experience is very common in America, perhaps the commonest member of the *A. glaucus* group. There is reason to regard the group as containing some members of fairly stable morphology and others which are variable in measurements and markings even of the ascospores, which are very frequently somewhat larger than 4.7 by 3.3μ.

Sartory (Champignons parasites de l'homme et des animaux, p. 581. 1922) attributes 8 per cent of the cases of otomycosis to this species. In contrast to Mangin's restriction of the name to forms with ascospores 4.7 by 3.7μ, Sartory includes the whole series with ascospores from 4 to 5.6μ in long axis under the name.

Perithecial forms which probably represented members of the A. glaucus group but which are not identifiable by the descriptions given, hence to be dropped. For convenient reference these are arranged alphabetically

E. chilense Montagne. Syll. Crypt. no. 919; Fl. Chil. VII, p. 476; cited by Saccardo Syll. 1: 27.

The citation of "perithecia globose yellow" suggests the *A. glaucus* group, but the description lacks the details required for actual identification.

E. coriorum Wallr. Flora Cryptogamica Germaniae no. 2076, in Compendium Florae Germanicae 4: 331. 1833. = *E. repens* De Bary fide Mangin (loc. cit., p. 364), who reports the ascospores to agree exactly with *E. repens*. The description certainly points to one of the *A. glaucus* group producing yellow perithecia, but no microscopic data are given by Wallroth to separate his material from other members of the group.

E. desmazieri Castagne. Pl. Mars. II, Sup., p. 56, 1851; also Berlese, Fungi Moricolae Appendice, p. 11. 1889.

As described by Berlese, this was a perithecial form, probably belonging to the *A. glaucus* group, but not sufficiently described to identify.

E. diplocystis B. and Br. Jour. Linn. Soc. [London], 14, tab. 10, fig. 55–56. 1875. Neither description nor figure would identify this

with any definite Aspergillus, although the specimen may have been the perithecia of some member of the *A. glaucus* group.

E. epixylon Kunze and Schm., D. Schw. no. 83; Wallr. Fl. Crypt. no. 2078. In Compendium Florae Germanicae 4: 331. 1833. As described, the colonies were at first yellow then fuscous red, perithecia were citrine, smooth, crowded, and it grew abundantly upon wood in Germany and Italy.

Culture: None.

This was evidently some member of the *A. glaucus* group.

E. fructigenum Link, Sp. Pl. Ed. 4, 6, p. 80. 1824. The meager data given indicate colonies dark brick red, with perithecia citrine, small, evidently a member of the *A. glaucus* group, but not more closely determinable. The name appears to have been based on specimens collected by Martins at Erlangen.

Culture: None.

E. sacchari Spegazzini, Anales del Museo Nacional de Buenos Aires 6 (ser. 2ª, t. III): 244. 1899.

Perithecia only described as sulphur yellow, 150μ in diameter, in loose cottony mycelium; asci 8 spored, 10 to 12μ in diameter; ascospores ellipsoid, hyaline, rugulose, massed in mucus, 4 to 6μ by 2 to 4μ.

Description lacks the essential characters necessary to separate this from the other forms of *A. glaucus* with yellow perithecia.

Conidial forms described only from mycotic infections of the human skin

A. fontoynonti Guéguen. Archives de Parasitologie 14: 177–192, 2 pls., 36 figs. 1910. Latin diagnosis p. 192; see also Compt. Rend. Soc. Biol. [Paris] 66: 1052. 1909. Fungus isolated from an abcess originating in Madagascar; mycelium uncolored; stalks smooth, septate, thin-walled, 150 to 200 by 3 to 5μ at base enlarging to 14 to 18 at the tip of the vesicle, which is not sharply defined; sterigmata 8 to 12 by 2μ, in one series but often growing out into secondary stalks bearing small heads; conidia ovoid at first smooth then sparsely aculeate, glaucescent, in age cinereus, 4 to 6 by 3 to 5μ, with chains crowded into an obconical mass; cultures not pathogenic to rabbits, guinea pigs or fowls.

Culture: Guéguen; not seen by us.

The figures and description place this form unquestionably with the *A. glaucus* group.

A. foutoynonti Guéguen, in Saccardo Syll. 22: 1256. 1913. Incorrect citation for *A. fontoynonti*.

A. tokelau Wehmer. Der Aspergillus des Tokelau. Centralbl.

Bakt. Abt. 1, 35; 140. 1903; Dubreuilh M. W. Jour. Méd. Bordeaux 32: 312. 1902.

Wehmer's description was based upon fixed pathological material, not upon culture. The description clearly points to mixed infections rather than the agency of this organism in pure culture. As described: Stalks varying greatly from short 100 to 200μ to long and coarser forms up to 900μ long, more or less sinuate, with walls thin, smooth, and colorless, bearing heads varying from 8 to 12μ in diameter to 30μ or occasionally 100μ in diameter; vesicular area not sharply marked, but merely the enlarged apex of the stalk 5 to 30μ in diameter; sterigmata 5 to 9 by 2 to 3μ, sometimes closely packed on the tip of the vesicle again becoming almost radiate, forming heads figured as resembling *A. glaucus* or rarely almost like *A. fumigatus*, usually in one series, a few sterigmata growing out into hyphae and occasional branched forms; conidia resembling those of *A. glaucus*, globose or somewhat elongated, very variable in size 3 to 12μ in long axis, marked with fine colorless spines, not resembling the conidia of *A. flavus*.

Culture: Not reported, only pathological material described.

The smooth thin walls of the stalk, single sterigmata and conidial markings ally this organism with the *A. glaucus* group. Beyond such grouping, closer identification must await cultural study. Sartory (Sartory, A. Champignons parasites de l'homme et des animaux, p. 520. 1922) refers to this species as *A. lepidophyton*.

Tribondeau in 1901 and 1903 (Tribondeau, Le lépidophyton, champignon parasite du Tokélau, in Compt. Rend. Soc. Biol. [Paris] 53: 53–56, 2 pls., 1901, ibid. 55: 104–105. 1903) called the mold parasite of tokelau "lepidophyton" stating that it was an Aspergillus. Wehmer (Centralbl. Bakt., abt. 1, 35: 140. 1903) assigned to "Tribondeaus Lepidophyton als species namen *Aspergillus Lepidophyton* oder wohl besser *A. tokelau*" and describes it under the latter name, and is quoted by Pinoy, in Bull. d. l'Institut Pasteur 1: 822, 1903 as using the term *Aspergillus lepidophyton*.

Conidial forms described in terms indicating membership in the A. glaucus group, but few, if any, of them are identifiable. For convenience these names are arranged alphabetically here; an analysis based upon details of color, size or occurrence is proposed in the Key. Unless further studied these names may be regarded as belonging to the doubtful synonymy of the group, hence most of them may be dropped without serious consideration

A. argentinus Speg. (Spegazzini, C. Hongos de la Cana de Azucar. Rev. Fac. Agron. Veter. La Plata 2: 245. 1896.)

Essential data from the Latin diagnosis and from the describer's notes in Spanish: Growth sparse or in loose masses on culms of partially dead and fermenting grasses, and differing from *A. glaucus* in color and especially in the smoothness of the conidia, at first white then gray; sterile hyphae creeping, up to 5 to 7μ in diameter; stalks 250 to 2000μ by 10 to 15μ, sparse or clustered, erect, firm, continuous (?) hyaline; vesicle 35 to 40 by 30 to 40μ; sterigmata 8 to 10 by 4μ; conidia globose, 6 to 10μ in diameter, hyaline, smooth. Perithecia not reported.

Culture: None.

Separation based upon smooth conidia and gray color is scarcely adequate to identify a member of this great group growing under conditions manifestly unfavorable. Unless the organism should be rediscovered and studied further, the name must be dropped.

A. brunneofuscus Sée. (Sée, Pierre. Les Maladies du papier piqué. p. 29. Paris. 1919.)

Colonies growing rapidly on usual media, very brown to almost black with mycelium reddish brown; conidiophores, septate, erect, occasionally branched 150 by 10 to 15μ; vesicle ovoid to globose 35 to 40μ diameter; total head with spores up to 75μ diameter; sterigmata simple, on summit of vesicle, 20 to 25 by 6 to 8μ; conidia echinulate, globose up to 15μ, or ovoid 12 to 18 by 8 to 15μ.

From the description as given this species probably represents one of the *A. glaucus* group in which reddish brown pigment produced in the substratum and deposited in and as an incrustation upon the cell-walls has masked the green color of the scantily produced heads. Some of these forms produce perithecia tardily and under special cultural conditions which would not be presented in Sée's material as described. It is very doubtful if a species peculiar to paper will be finally identified.

Culture: Sée.

A. caesiellus Saito. Jour. Coll. Sci. Imp. Univ. Tokyo 18 (5): 49, pl. III, fig. 14. 1904.

Colonies persistently gray to green, stalks very short with wall smooth and thin, 100 to 200 by 4 to 6μ; vesicle flask-shaped 12 to 14μ in diameter; sterigmata up to 12 by 3μ in one series, few in the head; conidia, 4 by 7μ, mostly elliptical or oval, smooth.

Cultivated: By Saito; not seen by us.

A. carneolus Sacc. Michelia 1: 77. 1877, and Fungi italici, no. 18.

Reported only once on culms and panicles of rotting sorghum; colonies dirty flesh-color (sordide carneus); heads figured as radiate; stalks up to

120 to 130 by 10μ, with prominent septa; vesicles up to 30μ diameter; conidia ovate-oblong 6 to 8 by 3 to 4μ, hyaline to rosy.

Culture: None.

While not identifiable from the description given, this was probably some one of the *A. glaucus* series. Colony color is readily accounted for in this group by encrustment of hyphae.

A. cinerescens Bainier and Sartory. Bul. Soc. Myc. France 27: 98–104, pl. III, figs. 6–12. 1911.

Stalks up to 125μ long, gradually enlarging upward to 14μ at the apex where the sterigmata are borne without a definite vesicle; sterigmata of the *A. glaucus* type forming a closely packed verticil with chains of conidia forming a solid column; conidia elliptical to globose, 2.8 to 5.9 by up to 11.2μ, or 2.8 to 11.2μ, slowly dark gray, figured as smooth.

Culture: By Bainier and Sartory; not identified by us.

A. delacroixii Sacc. and Sydow. Sylloge fungorum 14: 1044. Syn. for *A. olivaceus* Delacr., Bul. Soc. Myc. France 13: 118. 1897.

If the genus Aspergillus is to include Sterigmatocystis, the name *A. delacroixii* becomes untenable because of *S. delacroixii* (Delacr.) Sacc., but until this species has been reisolated and its separateness from other members of the *A. glaucus* group has been fully established, further correction of nomenclature is not desirable.

A. ferrugineus Link. Sp. Plant. Ed. 4, 6, pt. 1, p. 68. 1824. = *A. ferrugineus* Fries, Sys. Myc. 3: 387. 1829. Link and Fries appear to have had the same data. The note that this is a smaller form than *A. glaucus* establishes a presumption that this form was one of those members of the *A. glaucus* group with hyphae richly studded with reddish granules, giving a brown colony, but leaves the identification of a particular member of the series impossible. Durieu and Montagne commenting upon the identification and relations of *A. phaeocephalus* found *A. ferrugineus* unrecognizable at that time (1849).

Culture: None. To be discarded.

A. giganto-sulphureus Saito. Unters. atmosph. Pilzkeime, Jour. Coll. Sci. Imp. Univ. Tokyo 18 (5): 48, pl. III, fig. 12a to d. 1904.

Colonies colorless then yellow, finally brownish yellow; stalks with smooth wall, colorless, 1 mm. or more in length and figured as septate; sterigmata in one series 24 to 28 by 7μ, in a terminal group only; conidia subglobose 8 to 12μ, smooth or rough; perithecia not found.

Culture by Saito; not seen by us.

Saito's figures taken with the description suggest placing this in the

A. glaucus group, but its real affinity may have been with *A. oryzae*, as suggested in our previous paper (Thom and Church, Am. Jour. Bot. 8: 107. 1921).

A. glaucus var. *albida* Speg. An. Mus. Nac. Buenos Aires 6 (Ser. 2, t. III), p. 332. 1899. Separated as a "type with color white and a little larger" on dead branches. Not identifiable.

Culture: None.

A. glaucus variety *clavatus* is cited by Wehmer and attributed to Chevallier (Chevallier, F. F. Flore des environs de Paris I: 63, 1826). Under *A. glaucus*, this author gives three varieties, α. fungorum, olivaceo-griseus, capitulis majoribus; β. grisea, minima, dense grisea aut cinerascente glauca and γ. clavata, albida, capitulis clavatis, but without other data for distinction, and evidently without regarding his varieties as having taxonomic standing.

A. glaucus var. *minimus* Hanzawa. Jour. Coll. Agr. Tohoku Imp. Univ. Sapporo 4 (5): 220, 1911. Described from dried fish on which a conidial form only is reported.

Colonies white, bluish white to clear green and later grayish or greenish brown; stalks thin-walled (−0.5μ), hyaline, smooth, 500 to 1000 by 4 to 16μ; heads large; vesicles globose or oval, 14 to 55μ, commonly 40μ; sterigmata 6 to 15 by 4 to 7μ. radiating; conida 6 to 17 by 6 to 12μ, thick-walled, smooth at first then granular.

Culture: By Hanzawa.

A. glaucus oblongisporus E. & E. Field Mus. Bot. 1. p. 88. 1896. Listed by Millspaugh in "The Living Flora of West Virginia," p. 32; pub. in West Virginia Geol. Survey 5(A), p. 32. 1913. Conidia oblong, elliptical 5 to 7.5μ by 2.5 to 3μ. Apparently from material labeled *A. oblongisporus* E. & E., no. 760 in Nuttall's flora of Fayette Co., W. Va. (see p. 212).

Culture: None.

A. glaucus var.* *olivascens* Sacc., F. ital., no. 702; Michelia 2: 543; Sacc. Syll. 4: 64, and *A. glaucus* subsp. *olivascens* Sacc. Syll. 12: 38. distributed by Sacc. as no. 1376, Mycotheca italica; described from rotting mushrooms. Certainly one of the group, but examination of Saccardo's specimen No. 1376 and of 1375, labeled *A. glaucus*, shows little ground for separating this form. Saccardo gives: Heads olivaceous green; stalks 400 to 500 by 18 to 20μ; vesicles up to 60μ; conidia globose, 8 to 10μ; rough, olivaceous.

Culture: None.

A. glaucus var. *subolivaceus* Ferraris. Fl. Ital. Crypt. Pars. 1, fasc.

13: 911. 1914. The characters given are stalks 900 to 1500 by 8 to 17μ; vesicles 43 to 70μ; sterigmata 9 to 12μ; conidia 7 to 9.5 by 6 to 8.5μ.

Culture: Not reported.

A. macrosporus Bonorden. Handbuch Allg. Myk., fig. 193. 1851. The figure and description together with "black bread" as a substratum suggest one of the brown forms of the *A. glaucus* group, although Wehmer does not place it in this group, since no green color was noted by Bonorden.

Culture: None; not identified by us.

A. nanus Oudemans (Oudemans, C. A. J. A. Contr. Mycol. Pay. Bas., in Nederl. Kruidk. Arch. Deel 2, Ser. 3, p. 1121. 1904). Not *A. nanus* Mont. q.v. Described from parchment paper which had been smeared with marmalade:

Colonies with delicate superficial mycelium; stalks 180 to 235μ long, hyaline, continuous; vesicle nearly globose, hyaline, 20 to 25μ in diameter; sterigmata in one series 7 to 10 by $2\frac{1}{3}\mu$; conidia in long chains, radiating from the vesicle, globose or apiculate at one pole, at first hyaline then slowly fumose.

Culture: Not reported.

The specification that this colony grew upon a piece of paper used to wrap marmalade, the single series of sterigmata and the conidia pointed (apiculate) at one pole justify presumptively placing it in the series of names of conidial forms belonging to the *A. glaucus* group, which are not further identifiable without the discovery of their perithecia.

A. ochraceo-ruber Sacc. Michelia I, p. 77. Saccardo, P. A. No. 1063, in Mycotheca Veneta on bark of walnut 1876, see fig. 17 in Fungi italici.

The description of the colony suggests a large spored member of the *A. glaucus* group, with mycelium ochraceous to red, stalks up to 700 to 800μ long and conidia 15 to 18 by 12 to 43μ (13 in F. ital.). Material distributed by Saccardo (above) showed no conidia larger than 15 by 8 to 10μ in one packet, but in another conidia up to 33 by 21μ or larger, some stalks with smooth walls, some with pitted walls.

Culture: None; not recognizable definitely.

A. olivaceus Delacroix. Bul. Soc. Myc. France 13: 118–120. 1897. Syn. *A. delacroixii* Sacc. and Sydow, Sylloge fungorum 14: 1044. Not *A. olivaceus* Preuss, 1852.

Colonies at first white then olivaceous; mycelium hyaline; stalks erect, hyaline, ovoid, 8 to 15μ diameter; sterigmata hyaline 6 to 7 by

2.5μ, chains sometimes branched (?CT); conidia at first hyaline, then subglaucescent, finally pale olivaceous, ovoid, smooth 5 to 6 by 3.5 to 4μ, with connective.

Culture: Not reported. Described from beans of *Theobroma cacao*.

Saccardo correctly noted that this name was untenable on account of Preuss' *A. olivaceus*, but until Delacroix's form is isolated and restudied to determine its correct placing, further correction of nomenclature may be deferred. Delacroix's material probably belonged in the *A. glaucus* group.

A. penicillatus Grev. "Grev. Fl. Edin. ined." Greville Sc. Fl. 1: 32, pl. 32. 1823. Syn. *A. glaucus* (conidial). Described and figured from damp grasses in the herbarium, as cinereous with stalks scattered or gregarious, simple, $\frac{3}{4}$ of a line high, heads loose, somewhat drooping. The figure is a good sketch of a conidial form of the *A. glaucus* group as seen in herbarium materials many times. Without the ascosporic form, such material is not identifiable, hence the name must be discarded. This species is cited by Saccardo (Sylloge 4: 85) from Sturm as *Briarea elegans*, but the interpretation offered above appears more reasonable from the study of many such collections.

A. penicillatus Link. Sp. Plant. Ed. 4, 6, pt. 1, p. 69. 1824. The description given by Link is not sufficient to identify any form, but since he probably intended to cover the organism of Greville, it may be assumed to be conidial *A. glaucus*.

Culture: None.

A. rufescens Berlese (Berlese, A. N. Fungi Moricolae, fasc. VII, no. 4, tav. 54, fig. 12–17. 1889).

Colonies at first colorless then greenish red, finally brick red; stalks up to 1.5 mm. long, simple or branched at apex, figured as brown; vesicles subglobose, figured as brown; sterigmata in one series, each bearing three sterigmatic points from which conidial chains arise; conidia globose or ovoid, subhyaline with walls fairly thick, slightly roughened 10 to 12 by 10μ. Found upon living roots of *Morus alba*, but not cultivated.

If Berlese is correct in figuring sterigmatic points upon each simple sterigma, his species is not an Aspergillus. If his sterigmatic points are merely projections of the cell-wall due to rough handling in mounting for examination as we have seen many times in this group, his specimens belonged to one of the reddish brown strains of the *A. glaucus* group. The latter interpretation is most reasonable, but identification of the species is probably impossible.

A. sartoryi Syd., in Sartory, A., and Sydow, H., Ann. Mycol. 11: 156–160, pl. VIII. 1913.

Colonies in shades of orange-yellow, isabelline, to deep olive buff in age; stalks long, conspicuous, 12 to 13μ in diameter; vesicle 45 to 50μ in diameter, claviform to globose; sterigmata variable in size 15 to 20 by 7 to 9μ; conidia oval to globose, up to 9 to 10μ in long axis, in divergent (radiating) chains, presumably smooth (no record), "endogenous" (inner and outer wall more or less separate and "connective" more or less evident); perithecia none.

Culture: Sartory and Sydow, from the mines of Johannisberg.

The colors given would allocate the species to the group with *A. tamarii*; the morphology as described, assuming the stalks and conidia to be smooth (not described but so figured), put it in the *A. glaucus* group.

A. spiralis Grove (Grove, W. B. New or noteworthy fungi, Jour. Bot. 23: 164, tab. 257, fig. 5. 1885).

Colonies described as occurring on the cork of a bottle of carminate of ammonia, with hyphae dichotomous, interwoven, citrine in color, with spirals; stalks yellow, erect, unseptate, once or twice dichotomous; sterigmata in one series 20 to 30 by 10μ, more or less constricted in center; conidia obovate to globose, unequal, smooth, yellow, 8 to 15μ, mostly 10 to 12μ in long axis.

Culture: Not reported; never identified after Grove's description; to be discarded.

The substratum, a cork stopper in a bottle of either carmine stain or red ink (?), together with the size of the conidia and the spirally wound hyphae point to a conidial form of the *A. glaucus* group, with the aborted coils which would upon favorable substrata produce perithecia. The yellow stalks and conidia might easily represent a staining effect from the solution. Tentatively placed in *A. glaucus* group.

A. stercoreus Sacc. Michelia 1: 78. 1877; also Fungi italici, no. 19.

The description and especially the figure in F. italici suggests an organism of the *A. glaucus* group, with floccose, canescent colonies, but without perithecia; stalks 600 to 800 by up to 20μ, erect, unseptate; sterigmata oblong-conical, covering the vesicle; conidia ovoid to globose, hyaline, 7 to 10 by 6 to 7μ. Exsiccati: No. 1061 in Mycotheca veneta, specimen in the Pathological Collections of U. S. Dept. of Agr. destroyed, no Aspergillus found in it.

No record of study beyond the examination of the original material is to be found, the name can not, therefore, be maintained.

A. virens Link. Obs. p. 16. 1809; Sp. Pl. ed. 4, 6, pt. 1, p. 67. 1824. "Caespitibus densis, floccis intricatis virentibus erectiusculis, capitulis concoloribus. Caespites late extensos format, floccis dense intricatis. Capitula magna, sporidiis, globosis seriatis. In sebo, quod rebus conditis infundi solet."

The original description quoted from Link's Observationes appears with minor variations in his Species Plantarum, in Fries' Systema Mycologicum 3: 388, and in Sprengel (Sys. Veg. 4, ed. 16, p. 541. 1827). The substratum named as the fat in food cooked with fat and stored, would suggest some member of the *A. glaucus* group, but furnishes no means of indicating which form was observed.

Saccardo, in fig. 20 of Fungi italici and in Michelia I: 78. 1874, believes he has Link's species from wasp's nests, and describes the stalks as 300 to 500μ in length by up to 10μ in diameter, sparingly septate, with heads and conidia greenish. The heads are figured as radiate. Conidia 3μ in diameter. In age the sterigmata and chains of conidia fall away, leaving the naked vesicle. Although not identifiable from either the description of Link or of Saccardo, some members of the *A. versicolor* group might satisfy the description.

Eichelbaum (Verh. Naturw. Ver. Hamburg 3 Folge, XIV (1906): 34. 1907) applies the name to African material from a putrescent Xylaria which produced hemispherical perithecia about 120μ in diameter at the base and about 90μ in height, greenish yellow; ascospores smooth, round, 4μ in diameter; stalks 2 to 2.5 mm. in length, 25μ in diameter at the apex; conidia globose, smooth, 4.3μ in diameter. The shape of the perithecia and ascospores excludes the *A. glaucus* group and, if literally correct, excludes *A. nidulans*. This description is cited by Saccardo in Sylloge Fungorum 22: 1255. 1913 in proposing the name *Eurotium virens* (Eichelb.) Saccardo.

Van Tieghem (Bul. Soc. Bot. France 24: 96, 167, 206. 1877) uses the same for a colony found upon leather and differing in color from *A. glaucus* and *A. repens* which were also present.

Any attempt to place cultures under this name by comparison with either discussion would be purely speculation, although the organism may have belonged with *A. versicolor* or *A. penicilloides*.

Culture: None; not seen by us. The name should be dropped.

SECTION IV

Intermediate Forms

Conidia mostly less than 5μ in long axis

1. Some described in terms indicating affinities with the *A. glaucus* group, others of unknown affinities (compare p. 101).
   - a. Perithecia described as yellow..... *A. subgriseus* Peck
     A. belfanti Carbone
   - b. Perithecia not reported.
     Conidia elliptical or barrel-form, heads dry, very delicate species
     A. gracilis Bainier
     Heads enveloped in slime when ripe..... *A. conicus* Blochwitz
   - c. Conidia globose, 4 to 4.5μ..... *A. penicilloides* Speg.
     A. brunneo-virens Delacroix
     A. cinereus Speg.
     Conidia globose, 3 to 4μ..... *A. varians* Wehmer
     Conidia globose, 3μ..... *A. koningi* Oudemans
     Conidia oval, 2μ..... *A. minimus* Wehmer
     Conidia globose, 1μ..... *A. pusillus* Massee

A. subgriseus Peck. Bul. Torrey Bot. Club 22: 210. 1895. Compare *E. subgriseum* Peck. Conidial areas gray or bluish gray, originally described upon Corticium; stalks erect, continuous 100 to 125 by 7 to 8μ; vesicle subglobose 30 to 40μ, bearing globose, hyaline conidia 3.5 to 4μ.

Culture: None. Peck in his description of *E. subgriseum* assumes that he had the ascosporic form of his original species.

Eurotium subgriseum Peck. Rept. N. Y. State Mus. Bot. 1910, p. 30. 1911 (N. Y. State Mus. Bul. 150. 1911.) Compare *A. subgriseus* Peck. An ascosporic form of the *A. glaucus* group described from dead wood and bark; perithecia pale yellow, densely clustered, subglobose, 100 to 125μ, with ascospores greenish-yellow in mass, globose 6 to 8μ in diameter.

Peck assumed without culture the identity of this ascosporic form with his *A. subgriseus* described from conidial material. The description given does not describe the ascospores in sufficient detail for identification; probably both should be dropped.

A. belfanti Carbone. Atti. Ist. Bot. Univ. Pavia 14, Ser. 2, p. 321, fig. 11. 1914.

Carbone's ascogenous form has sulphur yellow mycelium bearing greenish sinuate stalks about 7 by 125 to 130μ, tipped by greenish vesicles pyriform to subglobose about 13.5 by 13.5 to 15μ with one series of sterigmata 3 by 4.5 to 5μ; conidia oval, olivaceous, about 5μ; perithecia yellow varying from 100 by 130 to 140 by 160μ, with asci 8 to 14μ diam., each containing 4 to 6 ascospores, smooth, hyaline and globose.

Culture: By Carbone, but without more complete information, the species can hardly be identified.

A. gracilis Bainier. Bul. Soc. Myc. France 23: 92, pl. IX, figs. 11–14. 1907.

Bainier has described a very delicate species verging toward a Citromyces (Penicillium) producing small blue-green or green colonies, with short delicate stalks 3μ in diameter at base broadening toward the apex into a vesicle in form of a truncated cone up to 25μ in diameter and bearing one series of sterigmata 5 to 6μ in length, with conidia at first barrelform then subglobose about 3μ in diameter in chains packed in a column. A culture showing approximately this morphology (no. 4246, now lost) was isolated from corn at Washington. This recognition of *A. gracilis* differs from Saccardo (Sylloge 22: 1255) who suggests relationship to *A. clavatus*.

A. gracilis var. *exiguus* Bainier and Sartory, Bul. Soc. Myc. France 28 (1): 47, pl. II. 1912. According to the description this variety differs in physiological characters slightly from *A. gracilis* Bainier.

A. conicus Blochwitz. Published by Dale, E. On the fungi of the soil, pt. II. Ann. Mycologici 12:38. 1914, described as a Penicillium, Ann. Mycol. 10: 465. 1912. We have cultivated Miss Dale's form and have independently obtained it or related organisms from several sources in America and Europe.

The following description is developed in broad enough terms to cover several of the strains we have cultivated. Colonies slow growing, forming a slimy, convoluted or buckled mass with submerged irregular margin, colorless then green to dark green or almost black; reverse of colony also dark green to almost black, with stalks and heads sometimes erect and free from slime at first but later enveloped in a mass of greasy slime; stalks short sometimes 100 to 200μ long; vesicles up to 20μ in diameter, fertile mostly at apex only; sterigmata of the *A. glaucus* type 5 to 10μ by 2 to 4μ; in some strains developing into secondary stalks bearing little heads; conidia elliptical, 4 to 6 by 3 to 3.5μ, in some strains smooth, in others up to 8μ in long axis, becoming thick-walled and rough as in *A. glaucus* in age. Perithecia not found.

Culture: Dale, Thom and Church, Nos. 2502 (lost), 4733.70.1, 4733.64.1.

The strains of this group are unique in their production of masses of slime, but agree with members of the *A. glaucus* group in other characters.

A. penicilloides Spegazzini. Rev. Agrar. Veter. La Plata, p. 246. 1896.

The species was described from specimens of moldy sugar-cane in Argentina, but was not cultivated by Spegazzini. Culture no. 4197.3, isolated by Owen from cane products in Louisiana, was sent to Spegazzini and accepted by him as probably identical with his species.

The following emended description is, therefore, offered. *A. penicilloides* Spegazzini emended Thom and Church: Colonies not growing or growing very poorly on Czapek's solution agar, growing readily upon cane syrup agar, dusky green, heads small, columnar (calyptriform); stalks up to 200μ long by 3μ at base, increasing to 5 to 7μ just below the vesicle; vesicle fairly abrupt, 8 to 12μ in diameter, sharply marked off from the stalk in cases by an actual constriction; sterigmata in 1 series packed as in *A. fumigatus* up to 8.5μ long and 2.5μ in diameter; conidia elliptical to globose, 3 to 3.5μ in diameter, rough, showing a connective, with walls dark or blackish. Perithecia not found.

A. brunneo-virens Delacroix. Bul. Soc. Myc. France 13: 120, with text figure. 1897.

Organism described as found between the cotyledons of peanuts; colonies powdery, becoming green or olivaceous from brown; mycelium sparse; stalks sinuous 200 by 7 to 7.5μ, constricted just below the vesicle; vesicle up to 10μ diameter; sterigmata in one series, cylindrical, hyaline or greenish; conidia globose or subovoid, smooth 4 to 4.5μ.

Culture: Not reported; not seen by us; perhaps near *A. glaucus* group, since in that group the superficial mycelium frequently forms a brown background for green conidial heads.

A. cinereus Speg. Spegazzini, C. Fungi argentini, Anales Sociedad Científica Argentina 10: 162–163. 1880.

Heads sparse almost solitary (cinereus), gray; stalks slender, unseptate, 200 to 250 by 3μ, colorless; vesicles 50 to 70μ; sterigmata cylindrical or conical, 8 by 3μ, colorless; conidia smooth, colorless 3.5 to 4μ in diameter. On dry plants in the herbarium.

The description given contains questionable measurements, such as vesicles 50 to 70μ upon stalks 3μ in diameter. It is doubtful if any organism corresponding with these figures will be found. If the meas-

urement given for the vesicle be applied to the whole head, some strains allied to *A. penicilloides* might satisfy the description.

A. koningi Oudemans, Arch. Néerl. 7, sér. 2, tab. 14, p. 284. 1902.

A small form isolated by Koning from Holland soil; with stalks up to 350μ, long, straight or flexuous, unseptate, hyaline; vesicles hyaline, 16 to 20μ in diameter; heads radiate; sterigmata in one series 8 to 10 by 2.5μ; conidia globose, 3μ, cream color, smooth.

Culture: Koning, Oudemans. This organism has not been seen by us.

A. minimus Wehmer. Bot. Centralb. 80: 454–456. 1899. Wehmer Monogr. p. 79.

Colonies green to gray-green, to dark green or dirty gray; reverse not colored or grayish; stalk smooth, colorless, 300 to 500 to 1,000 by 6μ, with one series 5 to 7 by 3μ, radiate, deciduous in age; conidia oval, smooth, colorless, about 2μ in diameter.

Culture: By Wehmer; not identified by us.

A. pusillus Massee. Kew Bull. Misc. Inf. 4: 158. 1914. From the description this is a very small gray colony from soil in Africa, which would be readily distinguished from other members of the group. A relationship to the *A. fumigatus* series is possible.

"Maculae effusae, majusculae, griseae. Hyphae steriles, repentes, parce septulatae, hyalinae, longissimae; fertiles erectae, rectae, hyalinae, continuae, 50 to 75 by 3 to 4μ apice vesiculosoinflatae, 10 to 12μ diametro. Sterigmata cylindracea, 3 by 1μ. Conidia catenulata, globosa, levia, sub lente hyalina, 1μ diametro" Sudan. Soil near Khartoum, R. E. Massey, nos. 3 and 4, forming a somewhat large spreading patch on culture media. Very small in every way.

Culture: Massee; not seen by us.

INTERMEDIATE SPECIES OF DOUBTFUL RELATIONSHIP

A. varians Wehmer. Ueber einige neue *Aspergillus*-Arten (A. varians, A. minimus, A. ostianus), Bot. Centralb. 80: 460–1. 1899. Wehmer Monogr. p. 77–79, taf. I, 1. gives: Colonies a beautiful green, becoming darker and even brown in age; mycelium colorless at first, often yellow-brown in age; stalks 1 to 2 mm. long by 10 to 14μ in diameter, smooth, colorless, not crowded upon most media; vesicles 25 to 30μ diam., globose or oval, in age showing a rough wall and yellow-green contents; sterigmata unbranched 16 to 25 by 3 to 4μ, radiating from the whole surface of the vesicle; conidia globose, smooth or finely granular, 3 to 4μ in diameter; perithecia and sclerotia not found.

Culture by Wehmer.

Culture no. 115 received from the "Centraalstelle" at Amsterdam in 1910 under this name produced floccose mycelium, in reverse lemon yellow, with sparsely scattered stalks fringed with drops of crystal liquid and bearing large green heads which were more or less columnar; stalks 200 to 800μ by 8 to 17μ in broadest places, rather thin-walled, smooth, colorless, few (1–2) septate; vesicles 15 to 30μ in diameter; sterigmata in 2 series, primary 8 to 10 by 3 to 3.5μ, secondary 8 to 12 by 3 to 3.5μ in pairs or three usually; conidia yellow-green, broadly elliptical to globose 3.5 by 4μ to 4μ, at first colorless and smooth, then green and slightly rough. This culture was lost and no similar form has since been identified. This culture satisfied Wehmer's description fairly well, except for the sterigmata in 2 series, better probably than any culture of the *A. versicolor* group, which has been identified with this species by Tiraboschi (Annali di Botanica 7: 9–12. 1908).

SECTION V

The A. Fumigatus Group

HEADS GREEN, WITH STERIGMATA IN 1 SERIES, AND CONIDIAL CHAINS FORMING A COLUMNAR MASS

A. fumigatus Fresenius. Beiträge zur Mykologie, p. 81, pl. 10, figs. 1–11. Frankfurt, 1850–53. See Wehmer, Monogr. p. 70. 1901. Perithecial form described by Behrens, J. Centralbl. f. Bakt., etc., 11: 335. 1892; by Grijns, Centralbl. f. Bakt. abt. 2, 11: 330. 1903; and by Thom and Church, Am. Jour. Bot. 5: 92–94, 1918. Vuillemin, Arch. Parasit. 8: 540. 1904, decides that asci have never been found in *A. fumigatus* and that Grijns was dealing with *A. pseudo-nidulans*, while Behrens had some strain of *A. glaucus*. Fresenius did not cultivate his organism but his figures and description fix the general characters of this whole group of forms which are cosmopolitan both as saprophytes and as parasites, especially in birds. We have examined hundreds of these strains in culture and as exsiccati. The group was characterized by Thom and Church (loc. cit., p. 93 see also fig. 13 and plates II and III, 118) as follows:

Colonies on Czapek's solution agar in some strains strictly velvety, in others with varying amounts of tufted aerial mycelium up to felted floccose forms, green to dark green, becoming almost black in age, spreading. Reverse and substratum, in some strains uncolored, in others showing varying amounts of yellow, this occasionally becoming reddish in age. Conidiophores short, usually densely crowded, up to 300μ (occasional strains to 500μ) long by 2 to 8μ in diameter, frequently more or less green colored, especially in the upper part, arising directly from submerged hyphae or as branches from aerial hyphae, septate or unseptate, gradually enlarged upward, with apical flask-shaped vesicles up to 20 to 30μ in diameter, fertile mostly only on the upper half, bearing sterigmata in one series, usually about 6 to 8μ (varying from 5 to 10μ) by 2 to 3μ, crowded, closely packed with axis roughly parallel to axis of the stalk, chains of conidia form solid columns up to 400μ by 50μ, but usually much shorter; conidia dark green in mass, globose, 2 to 3.5μ, mostly 2.5 to 3μ in diameter. Cultures grow well at 37°C. or higher.

In their characterization of the group, Thom and Church included in

A. fumigatus Fres. the description of the perithecial form later recognized as *A. fischeri* Wehmer. This form is certainly closely related to *A. fumigatus*, but there is a very abundant and variable series of forms with the characters of *A. fumigatus* Fresenius in which persistent search and experiment has failed to find an ascogenous phase. It has been deemed better, therefore, to separate these two sections of the group in this paper.

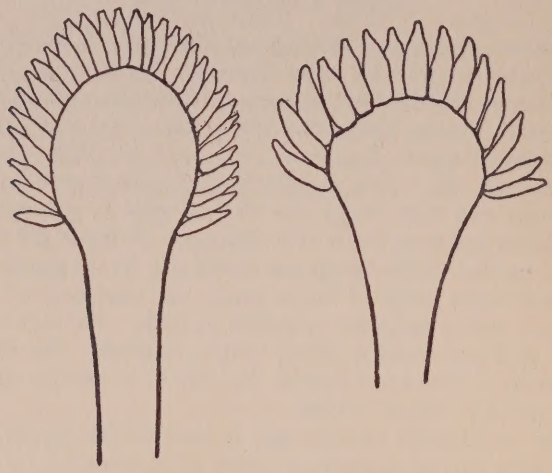

FIG. 13. A. FUMIGATUS, FROM STRAIN 118

Outlines and proportions determined with the camera lucida; sterigmata are diagrammatic. These optical sections showing marked variation in the same culture are introduced to illustrate the fallacy of specifying the proportion of the vesicular head of the stalk covered by sterigmata as a major character in separating members of this group into species. The details of measurement both of vesicle and sterigmata differ with the size of the head and the effect of crowding among the sterigmata.

Cultures with the general morphology of Fresenius' species have been received from students of soil bacteriology in widely separated lands as one of the most abundant molds encountered. We have isolated them many times from cereal products and from unmilled grain, hay and other stock feeds. They are not so common in studies of the acid fruits. As an agent of disease, *A. fumigatus* is constantly encountered as a cause of lung lesions in birds, occasionally in man and other mammals,

and has been reported occasionally from the human ear, but not definitely shown in skin lesions.

James (unpublished work in the Bureau of Chemistry) has shown *A. umigatus* to be both thermophilic and thermogenic. It appeared repeatedly in heating experiments where other species were eliminated by the high temperatures produced and acting upon cracked corn in pure culture brought the mass to 52°C.

1. Perithecial forms close to *A. fumigatus*.
   Ascospores 3 to 3.5μ, rough..... *A. fumigatoides* Bainier and Sartory
   Ascospores 4.3μ in diameter, globose, smooth, see p. 123
   A. virens of Eichelbaum
   Ascospores 4 to 4.5μ, with a single equatorial band or frill
   S. pseudo-nidulans Vuillemin
   Ascospores 6 to 8μ, in long axis..... *A. malignus* Lindt
   Ascospores 7 by 4μ, with two equatorial bands
   A. fischeri Wehmer

A. fumigatoides Bainier and Sartory. Bull. Soc. Myc. France 25: 112, pl. 5. 1909; Compt. Rend. Soc. Biol. [Paris] 66: 22. 1909. See also Sartory, A. Champignons parasites de l'homme et des animaux, p. 573. 1922; and as Syn. *E. fumigatoides* (B. and S.) Sacc. Syll. 22: 1255. 1913.

The conidial apparatus described closely resembles *A. fumigatus:* Conidiophores 150 to 310μ, commonly sinuate (tortuaux), gradually enlarging from 5 to 6μ in diameter at base to a vesicular apex 31 to 35μ in diameter; sterigmata colorless, 8 to 14μ in length, sometimes on the apex only, sometimes covering the whole vesicular area; conidia dark olive green, oval 2 to 3 by 2μ. Perithecia produced on all common media, commonly in 8 or 9 days developed from hyphae which first coil up without septation, then become septate ascogones, which enlarge and eventually become a perithecium 65 to 92μ in diameter, with asci 20 to 26 by 12 to 18μ and ascospores 3 to 3.5μ rough. The description of the origin and histology of the perithecia follows closely that of *A. repens*.

Culture: Bainier and Sartory. This organism has not yet been seen by us.

In animal experiments, *A. fumigatoides* attacked rabbits, whose serum would agglutinate spores of this species, but not those of *A. fumigatus*. In fermentation experiments, saccharose and maltose were reduced, but lactose and glucose were not changed, starch was saccharified.

S. pseudo-nidulans Vuillemin. Arch. Parasitologie 8: 540–542.

1904. Vuillemin transfers the ascosporic form described by Grijns as *A. fumigatus* in Centralbl. f. Bakt., etc., 2 abt., 11, p. 330. 1903, to this specific name, emending Grijns' description by indicating the double nature of the band by which he separates his form from *A. nidulans* as described by Eidam. The discussion by Vuillemin tallies with the commonest of our American soil forms of *A. nidulans*, but not with the description of Grijns. Grijns describes the conidial apparatus as having sterigmata in 1 series 9 to 18μ in length (shown also in his figures); ascospores 4 by 4.5μ with a single band or frill about equatorial region, red in acid and blue in alkaline condition. The color reactions are those of *A. nidulans;* the conidial apparatus described belongs with *A. fumigatus*. This combination has not been reported by others.

Culture: Grijns; not reported by others.

A. malignus Lindt. Arch. Exp. Path. Pharmakol. 25: 257–271, figs. 1–11. 1889.

Lindt gives the following: Stalks up to 1,000μ long; vesicles 22 to 24μ in diameter; sterigmata 10 by 4 to 4.5μ covering the upper two-thirds of the vesicle, more or less divergent; conidia 3 to 4μ in diameter; perithecia developed as in the *A. glaucus* group, producing ascospores on bread in 7 days, surrounded by pseudoparenchyma, of woven hyphae, white, 40 to 60μ in diameter, ascospores lens-shaped or biconvex, 6 to 8μ in long axis, with double bands or frills and some markings on surface of the valves.

Culture: By Lindt; not found by us.

Lindt cultures *A. malignus* as obtained from the human ear and reports his cultures as pathogenic to puppies in which extensive lesions were produced; when grown upon bread, ascospores were produced quickly and abundantly, giving measurements and markings differing only slightly from *A. fischeri* of Wehmer. The conidial form was, however, very much larger than the typical *A. fumigatus*.

No culture seen: *A. fumigatus* group.

A. fischeri Wehmer. Centralb. f. Bakt., etc., 2 abt., 18, pp. 390–2, figs. 1–5. 1907. Syn. *A. fumigatus*—ascosporic, see Thom and Church, Am. Jour. Bot. 5: 91, 92. 1918.

In our previous paper cultures 4188.21 and 4190 were reported as an ascosporic form of *A. fumigatus*, we have since received no. 4651.2 from Dr. Wehmer as *A. fischeri*, which repeats the morphology of our nos. 4188.21 and 4190 obtained in cultures here.

The conidial form is not distinguishable in morphology from *A. fumigatus*. In culture, stalks and heads are sparingly produced at room

temperatures but abundantly produced at 37°C. Perithecia are quickly and abundantly produced in Czapek's solution agar with cane sugar, upon soil and other media. They are commonly up to 300μ in diameter, not colored, or very pale salmon, with walls scarcely colored, consisting of a single layer of cells, crushing easily, covered by a loose network of uncolored sterile hyphae; asci abundant, filling the perithecium within a few days, from 8 to 10μ by 10 to 12μ, subglobose, breaking down quickly to leave the perithecium full of ripe ascospores; ascospores biconvex, 7 by 4μ, consisting of a central body 5 by 4μ, with two frilled equatorial bands about 1μ in width and 3 to 4 similar but narrower and anastomosing bands on each convex surface, separating into 2 valves in germination.

Culture: No. 4651.2 received from Wehmer in 1923 is apparently identical with nos. 4188.21 and 4190, obtained in American cultures.

Names given to conidial forms mostly clearly representing strains of the A. fumigatus group, to be dropped except possibly certain strains separable by relative pathogenicity as indicated in the text

A. fumigatus var. *alpha* Sion and Alexandrescu. (Sion, V., and Alexandrescu, N. Sur la toxicité d'un type d'Aspergillus fumigatus isolé du maïs avarié. Compt. Rend. Soc. Biol. [Paris] 64: 288–289. 1908.) This variety did not grow at 37°C., but proved very toxic to experimental animals. No other basis of separation was proposed.

A. fumigatus var. *tumescens* Blumentritt. Ber. Deut. Bot. Ges. 23: 419–427, pl. 19, figs. 5, 6, 18, 19, 20, 21. 1905.

The culture described produced a dense, buckled, pseudo-parenchyma-like felt of mycelium with conidial masses not differing to any significant degree in measurements from *A. fumigatus*. Secondary heads from the outgrowth of sterigmata, branching stalks and septate stalks are figured. It is probably correctly characterized by the author a "culture cripple," since the differences are such as occur very commonly as a result of some unfavorable condition of growth.

Culture: Blumentritt; not separated by us.

A. fumigatus var. *minimus* Sartory (Sartory, A. Étude d'un Aspergillus du groupe fumigatus, A. fumigatus var. minimus. Bul. Acad. Méd. Paris ser. 3, 82: 304–305. 1919); isolated from sputum of a patient supposed to be tuberculous.

Mycelial hyphae 2 to 3μ in diameter forming a close felt; stalks erect, slightly sinuous, 100 to 150μ long by 4 to 5μ at the base, with walls fuligineous gray more deeply colored near the apex, enlarging gradually

to a sphaeroidal vesicle 20 to 25μ in diameter and fertile only on the upper half or two-thirds; sterigmata 5 to 8μ fuligineous; conidia globose or rarely elliptical 2 to 3μ, bronzed.

Colonies grew best at 37°, ceased growing at 42°C., flourished on all the usual laboratory media, and proved pathogenic to the rabbit and guinea pig. Although the author tabulated the differences of this variety from *A. fumigatus*, a study of many strains would show gradation obliterating any gap so completely as to make recognition impossible.

A. cellulosae Hopffe. Centralb. f. Bakt., etc., 1 abt., 83, pp. 531–37. 1919. Syn. *A. fumigatus* var.

Described from German soil as a cellulose destroying organism: Colonies dark smoky green: reverse colorless to greenish; heads becoming columnar masses of conidia, spreading somewhat toward the apex; vesicles up to 30μ diameter with nearly hemispherical sterigma bearing areas; sterigmata as in usual types of *A. fumigatus;* conidia globose 2.5μ, showing very delicate spinulose points.

Culture: No. 4474.1 received from Neuberg as this species is *A. fumigatus*. The constant presence of *A. fumigatus* in soil has been proved by our own observations and comparison of cultures received from many sources. Waksman (personal communication) finds members of this group able to decompose cellulose, but does not regard them as important agents of decomposition in the soil.

A. lignieresi Cost. and Lucet, Ann. Sci. Nat. Bot. (IX) 2: 137. pl. 5, figs. 19–23. 1905.

This culture from the lung of a penguin differs in cultural details from typical *A. fumigatus*, especially by the presence of swollen groups of cells in the mycelium; stalks arising from complex masses of aerial hyphae, green only in the upper part, undulate and non-septate, 180 to 230μ by 6 to 8μ, with vesicles up to 24μ in diameter; sterigmata about 6μ in length; conidia 2.4 to 3μ; temperature range 15° to 53°C. In laboratory experiments pathogenic to rabbits and fowls.

Culture: By Costantin and Lucet, who find members of the *A. fumigatus* group separable only by pathological experiment.

A. pulmonum hominis Welcker,[1] Kuchenmeisters Parasiten II, p. 144. This is discussed and figured by Theodor von Dusch, in Virchow's Archiv. (n. F. 1) 11: 561–566. 1857, but no ground is given for separating it from *A. fumigatus*.

A. bronchialis Blumentritt. Ber. Deut. Bot. Ges. 19: 442–446, pl. 22,

[1] Not seen.

figs. 1–6. 1901; also ibid. 23: 419–427, pl. 19, figs. 1, 3, 6, 7, 8, 19 and 23. 1905.

The colony is described as floccose in contrast to the commoner forms of *A. fumigatus*, which are velvety or produce very little aerial mycelium. Close relationship to *A. fumigatus* is evident, but separation was based upon floccosity in the mycelium and the presence of vesiculose mycelial cells in old cultures. The conidia were reported as 2.8 to 3.6μ, but many strains of *A. fumigatus* show this range in diameter.

Culture: It was isolated from the bronchi of a diabetic by Blumentritt and showed an optimum of 32°C. In laboratory experiments, cultures were not pathogenic to dogs and guinea pigs, but caused death of hens and pigeons.

A. virido-griseus Cost. and Lucet. Ann. Sci. Nat. Bot. (IX) 2: 140. 1905. The describers find this form to be pathogenic to rabbits not to fowls, and to be floccose whereas *A. fumigatus* is pathogenic also to fowls and is not floccose.

As described: Colonies very floccose, areas partly white, partly gray, cream gray, to dark green or dark gray green, but lighter than *A. fumigatus;* stalks thin-walled, septate, undulate, only slightly colored or tardily colored near the vesicle, 400 to 620μ long; vesicle 25 to 36μ diam.; sterigmata 5μ in length; conidia 2.8μ diam.

Culture: Costantin and Lucet.

We doubt if this strain could be picked with certainty from the great number of *A. fumigatus* forms, by the variations described; the name may be worth retaining for a floccose section of the group with the pathogenic characters mentioned.

A. aviarius Peck. N. Y. State Museum Rept. (1890) 44: 120, pl. 4, fig. 9–12. 1892. Synonym for *A. fumigatus*. This pathogenic form from the inner costal surface of a canary bird as described presents no characters to warrant separating it from *A. fumigatus*.

Culture: None.

A. ramosus Hallier. Zeitschr. Parasitenk. 2: 266–269, pl. 6, figs. 1–6. 1870. The figures and descriptions evidently represent a strain of *A. fumigatus*.

A. nigrescens Robin. Histoire Naturelle des Végétaux Parasites, p. 518, atlas, pl. 5, fig. 2, Paris. 1853. The organism of Robin has been called *A. niger* by Wilhelm (Beiträge zur Kenntnis der Pilzgattung Aspergillus, p. 70. Inaug. Diss. Strassburg-Berlin. 1877) and *A. fumigatus* by Siebenmann (Die Fadenpilze Aspergillus flavus, niger u. fumigatus; Eurotium repens (u. Aspergillus glaucus) and ihre Beziehungen zu

Otomycosis Aspergillina. Inaug. Diss. Wiesbaden. 1883) and is judged undeterminable from the information given, by Wehmer (Zur Kenntnis einiger Aspergillus-Arten. Centralbl. f. Bakt., etc., 2 abt., 18, pp. 394–395, 3 fig. 1907). Robin's figures represent *A. fumigatus* much more closely than *A. niger*. Since this form was described from pathological specimens only, it is probably impossible to determine the organisms actually involved.

Culture: None reported by Robin; to be dropped.

A. glaucoides Spring. Bull. Acad. Sci. Belg. 19: 560–572. 1852. The name without description was given to a colony which grew in an egg under experiment. The same form was afterward found in another egg. It is recorded as closely related to, if not identical with, the mold found in air sacs of birds, hence probably *A. fumigatus*.

A. syncephalis Guéguen. Champignons parasites de l'homme et des animaux, p. 165, Paris. 1904. Mycelium white to gray; stalks erect subflexuous, unseptate, fuligineus toward the apex, up to 300 by 7 to 8μ; vesicle subglobose, bearing sterigmata only on the tip; sterigmata cylindrical; conidia smooth, gray, subovoid, 2.5 by 3.3μ, in chains forming a cylindrical column. Accidental culture, growing poorly at 15° to 16°C., rapidly at 25° to 26°C.

Guéguen's figure and description place this clearly with the *A. fumigatus* group, separated only by the ellipticity of its conidia. Sartory in his book of the same name (Champignons parasites de l'homme et des animaux, p. 573. 1922) reports this species as displaying the habit or aspect of Syncephalis, hence the name.

SECTION VI

The A. Nidulans Group

Heads with 2 series of sterigmata
Perithecial
Ascospores not described; conidial areas light marine blue
A. polychromus De Mello
Ascospores "smoke-gray"..... *A. rehmii* Zukal
Ascospores purple, with equatorial bands..... *A. nidulans* group

A. polychromus De Mello (De Mello, F. Contribution to the study of the Indian Aspergilli. Jour. Indian Bot. 1: 158–161. 1920).

De Mello describes his species as at first white, then through gray to "light marine blue," and passing through "gris souris" (brownish) with a violet tan to chocolate brown; with stalks 112 to 650μ, averaging 275μ long by 3 to 6μ; vesicles more or less elliptical up to 25μ in length by up to 20μ in diameter; sterigmata, primary 5 to 10 by 3 to 4μ, secondary 5 to 8 by 3 to 4μ; conidia, 2.5 to 5μ "finely punctuated" (spinulose?) bluish green to chocolate. "Perithecia yellow with a greenish tone found only in cultures aged, at least, 2 months; 30 to 35μ in diameter." Ascospores are not mentioned.

Cultures requested but not obtained. Regarded as near *A. nidulans* or *A. sydowi*, probably nearest the latter.

A. rehmii Zukal. Oesterr. Bot. Zeitschr. 43: 160, pl. 11, 12, figs. 1–10. 1893. *S. rehmii*, Sacc. Syll. 11: 593. Zukal regarded this form as close to a *S. sulphurea* Fresenius. Others have regarded it as close to *A. nidulans*, but some of Zukal's figures do not support that placing. The description given by Saito (Centralbl. f. Bakt. etc., 2 abt., 17, p. 159) clearly places his (Saito's) organism in the *A. flavus* group, but this is certainly incorrect for *A. rehmii*. While we have not seen a culture of *A. rehmii*, there are strains in the *A. flavipes* group which produce abortive masses of yellow mycelium which suggest the description of Zukal's organism.

The data given by Zukal follow: Mycelium densely woven, at first yellow, later ocher yellow; stalks 400 to 500 by 5μ, smooth, at first sulphur then ocher yellow; vesicle elliptical oval, 30 by 20μ; sterigmata, primary 6 by 2 to 3μ, secondary 4 by 1.5μ; conidia roundish "polyhedral," yellowish, transparent, smooth, 2.5 to 3μ; perithecia with soft

walls, found on spoiling ground oak bark, associated with P. luteum; fruiting in mid summer. No sclerotia were found. Perithecia cleistocarpic, globose or flattened, black, opaque, smooth, brittle, 100 to 200μ in diameter, with wall of cells in regular rows surrounded by a fairly dense yellow mycelial "Hülle" or felt, consisting of hyphae 1.7 to 2μ in diameter, and terminating partly in sterile vesicular "twigs" partly bearing lateral sterigmata; asci short stalked in bushlike clusters, obovate, very delicate and evanescent, up to 6 to 7 by 4 to 5μ; ascospores 8, elliptical; epispore smooth, thick, translucent gray, about 5μ by 3.5μ.

As an ascosporic species, it automatically falls close to *A. nidulans* with the chance that a more accurate description would have shown it to have been a member of that series.

A. nidulans (Eidam) Winter, in Rabh. Krypt. Flora, Pilze. 2 Aufl. Bd. 1. Abt. II p. 62, 1887, syn. *S. nidulans* Eidam. Cohn Beitr. Biol. Pflanzen 3: 392–411, pl. 21, 22. 1883. A characteristic and cosmopolitan group with essentially these characters is discussed and described by Thom and Church (Am. Jour. Bot. 5: 95–97. 1918) in the following terms:

Group characterization of *A. nidulans:* Colonies on Czapek's solution agar, white to yellowish green, or fairly deep green, velvety to more or less floccose in purely conidial areas, becoming definitely floccose when perithecia are forming or in strains predominantly ascosporic, floccose, with conidium production much reduced and green color absent or nearly so; reverse and agar usually more or less reddish to dark red or reddish brown; conidiophores more or less flexuous with the walls colored in shades of cinnamon brown, septate or unseptate, commonly 50 to 100μ less often 200μ long by about 3 to 5μ in diameter, increasing gradually to a dome-like vesicle 7 to 15μ in diameter, bearing sterigmata in two series, parallel with axis of the stalk; primary sterigmata varying, 5 to 8μ by 2 to 3μ; secondary sterigmata 7 to 10μ by 2 to 2.5μ; conidia globose up to 3μ or 3.5μ, occasionally to 4μ in diameter, smooth or rough, greenish, in parallel chains adherent into a solid column 30 to 50μ in diameter and up to 100 to 200μ in length.

Perithecia becoming globose, up to 200 to 300μ in diameter surrounded by floccose white to gray mycelium, the branches producing, either terminally or in a terminal series, yellowish to cinnamon, globose cells up to 25μ in diameter with walls 4 to 5μ in thickness (the Hülle); perithecial walls thin, brittle, consisting of one or two layers of polygonal cells from pink to deep red, almost black, turning blue with the addition of alkali and red again with acid. Asci pink to purple, numerous, filling

the perithecia, 8-spored; asci and purple red ascospores varying in size with the race or species.

The following variations may be described:

1. *S. nidulans* Eidam. Asci 10.5 to 11μ in longest axis, ripening slowly over a period of many weeks; ascospores slightly oval, about 5 by 4μ, smooth with deep purple walls, separating into two valves in germination. A culture with this type of ascospores has been found by us among the soil forms isolated by Waksman in New Jersey.

2. *S. pseudo-nidulans* Vuillemin. Asci 9 by 14μ; ascospores 4 by 4.5μ, lenticular, with double equatorial bands, these being extensions near the edges of the valves of the ascospore. Vuillemin regards this as the form which Grijns reports as an ascosporic A. fumigatus. The double character of the equatorial band is so distinct that it is difficult to believe that Grijns failed to see it if present. The possibility of finding Grijns' organism with a single equatorial band therefore remains open.

We have three variations of this ascosporic series distinguished as follows:

3. No. 110, received from Dr. Westerdijk at Amsterdam. Asci ripening slowly a few at a time over a period of several weeks, 10 to 13μ in diameter; ascospores lens-shaped, about 4 to 5μ in diameter and 3 to 3.5μ in thickness with a plaited, folded, or wrinkled equatorial band as a free extension of the margin of each valve to a width of 1.5 to 1.8μ (the double equatorial band of authors).

4. No. 4110, from flax straw and the same, No. 131, from soil, shows perithecia full of ripe ascospores within a few days; the asci mostly break down quickly, leaving the perithecium full of free ascospores; ascospores measuring as in No. 110, except that the equatorial bands are 1μ or less in width.

5. No. 4138T11 differs from No. 4110 in the appearance of ridges and folds on the valves of the ascospore. It was obtained by Waksman from New Jersey soil.

Diplostephanus nidulans (Eidam) Langeron, Compt. Rend. Soc. Biol. Paris, 87: 343–345. 1922. Synonym for *A. nidulans* (Eidam) Winter.

Perithecia not described

Pathogenic species closely related to if not identical with *A. nidulans*

S. nidulans forme *cesarii* Pinoy

S. nidulans var. *nicollei* Pinoy

A. jeanselmei Ota

S. unguis Émile-Weil and Gaudin

Possible synonym *A. flavescens* Wreden

S. nidulans forme *cesarii* Pinoy. (Pinoy, E. and Masson, P. Mycétome du poumon chez l'âne. Bul. Soc. Path. Exot. 8: 11. 1915.) Cited by Sartory, A. Champignons parasites de l'homme et des animaux, p. 605. 1922. Ascospores 4.1 by 4μ; sterigmata often simple and inserted toward the base of the vesicle; conidia 3μ.

S. nidulans var. *nicollei* Pinoy, in Nicolle, C., and Pinoy, Sur un cas de mycétome d'origine aspergillaire observé en Tunisie. Archives de Parasitologie 10: 437–458, pl. XI. 1906; see also Compt. Rend. Acad. Sci. [Paris] 144: 396. 1907. As described, this form was isolated from a case of mycetoma, or Madura foot with very extensive invasion of the tissues by mold.

Cultures were recognizable as clearly closely related to *A. nidulans*, with stalks up to 800 by 4μ, and stalk walls colored; vesicles about 10 to 12μ in diameter; primary sterigmata 8 by 3μ bearing two to four secondary sterigmata 4 by 2.5μ; conidia 2 to 3μ in diameter, delicately roughened, somewhat green; sclerotia 50 to 300μ in diameter, brown, more or less imbedded in mycelium are described instead of perithecia and these are figured as surrounded by the typical "Hülle" cells of the species called chlamydospores by the authors.

A. jeanselmei Ota. (Ota, Masao. Sur une nouvelle espèce d'Aspergillus pathogène: A. jeanselmei n.sp. Ann. Parasitologie, 1: 137–146. 1923.)

Stalks from 80, or more commonly, 100 to 400μ long, and from 4 to 5μ in diameter at base, up to 8μ near the vesicle, which is 16 to 29μ in diameter, with walls thin, apparently (in figures) smooth, somewhat dark colored; sterigmata shown as either simple and *A. fumigatus*-like or double as in *A. nidulans*, about 4 to 10 by 2 to 3μ; conidia globose or ovoid from 3.5 to 6.5μ in diameter mostly 5μ or less, smooth or in very old cultures somewhat verrucose, greenish becoming reddish brown in several months. Description suggests a variant of *A. nidulans*.

Cultures: Ota; not seen by us.

Ota found this form as a parasite on the nails of a patient in the hospital in Paris. His description puts it as possibly an intermediate between *A. gratioti* Sartory and *S. unguis* of Émile-Weil and Gaudin. The former has strictly one series of sterigmata, the latter two series, while *A. jeanselmei* shows both forms of sterigmata.

S. unguis, Émile-Weil, P., and Gaudin, L. Arch. Méd. Exp. et Anat. Path. Paris t. 28, no. 5, pp. 463–465. 1919. Parasitic about the human nails, fruit observed in direct examination.

Cultures at first white, then green or chrome-green, becoming brown in

age; stalks simple, unseptate, about 250 to 300 by 5μ; vesicle elliptical or pyriform to globose 8 by 14 to 16μ or about 12μ in diameter; sterigmata, primary cylindrical 5 by 3μ, secondary about 6 by 3μ; conidia in original lesions brownish 3μ, in culture globose, minutely rough in age. Perithecia not found.

Culture: By Émile-Weil and Gaudin; optimum about 30°C.

The data given group this form between *A. nidulans* and *A. sydowi*, nearer the latter. Transfer of species name to Aspergillus may properly wait verification of the grounds of separating this form from nearly related organisms previously described.

A. flavescens Wreden. (Wreden, Robt. Die Myringomykosis Aspergillina und ihre Bedeutung für das Gehörorgan. St. Petersburger Medizinische Zeitschrift 13: 133–184. 1867; also note of Robt. Wreden presented by Robin in Compt. Rend. Acad. Sci. Paris 65: 368–371. 1867.)

This organism appears to have been studied in pathological material only, not in any form of culture media. Robin compared it with *A. fumigatus* in material which had been identified by Fresenius and decided that it was not that species. The small spores 2 to 3μ in diameter; stalks with upper portions yellowed; and in the figures both primary and secondary sterigmata, suggest *A. nidulans* or one of the nearly related series such as *A. sydowi* or *A. versicolor* rather than *A. flavus* as has been frequently supposed. Lichtheim, L. Ueber pathogene Schimmelpilze. Berliner Klin. Wochenschr. 19: 128, 147. 1882, certainly used the name for a member of the *A. flavus* group, but there is no evidence that his identification was correct. Since it is not possible to decide the matter definitely the name should be abandoned.

Culture: None; to be dropped.

SECTION VII

A. VERSICOLOR GROUP

A. versicolor (Vuillemin) Tiraboschi, Ann. Botan. [Rome] 7:9, 1908; Syn. *S. veriscolor* Vuillemin. Mirsky, B., in Thèse de Méd. Nancy, no. 27, p. 15 et seq. 1903. Sartory, A. Champignons parasites de l'homme et des animaux, p. 602, regards *A. versicolor* as belonging with *A. nidulans*.

Mirsky gives: Colonies generally green with variations of rose, salmon, brown or even white; stalks 300 to 350 by 3.5 to 4.35μ; vesicles hemispherical on the tip of the stalk becoming 10 to 12μ in diameter; sterigmata on the hemispherical vesicular area, in 2 series, primary 3 to 4μ, rarely 9μ by 2.5 to 3μ, secondary 3 to 6 to each primary and 5 by 1.75μ; conidia oval to globose 3 to 3.5μ in diameter, yellowish, delicately verruculose.

Culture: Mirsky, Vuillemin and many others; Mirsky's culture was obtained from sputum of a consumptive.

The group designated *A. versicolor* is probably the basis of many of the difficultly interpretable names in the literature as indicated in the Key. No description antedating Mirsky gave sufficient information to bring about its general recognition. The name as published by Mirsky is, therefore, accepted by us for the series within which such strains as may be identifiable by other names may be grouped somewhat as indicated in this Key.

CHARACTERIZATION OF A. VERSICOLOR GROUP FROM CULTURE (COMPARE PLATE III, 2743)

Colonies on Czapek's solution agar, at first white, passing through shades of yellow, orange-yellow, "buff" to pea green or sage green, differing with the strain and conditions of culture, with green color occasionally entirely suppressed to produce orange buff, to almost flesh colored colonies or strains; reverse and substratum from yellow through orange to rose or red, varying in rate and completeness of change with the strain and conditions of culture or in a few strains either persistently colorless or developing color only in traces or very slowly; surface growth in some strains consisting almost entirely of condiophores

(velvety), in others showing a marked development of floccose, sterile hyphae bearing more or less abundant conidiophores as short branches or combinations of colonies with initially floccose centers and almost velvety outer zones; stalks, when arising separately from the substratum, up to 500 to 700μ long by 5 to 10μ in diameter, with walls smooth, almost colorless, 1 to 1.5μ thick; heads becoming 100 to 125μ in diameter, usually subglobose to globose, occasionally more or less calyptrate; vesicles 12 to 20μ in diameter, occasionally globose, more usually flask-shaped, fertile on the upper two-thirds, with radiating sterigmata in 2 series; primary sterigmata 3 by 5μ to 3 by 8 to 10μ, secondary sterigmata 1.5 to 2μ by 5 to 10μ, according to the variety and conditions of culture; conidia, globose, usually delicately roughened, 2.5 to 3μ, less commonly 3.5 or even 4μ, in diameter, usually in loosely radiating chains. Colonies digest milk, liquefy gelatin and in Czapek's solution produce a wine-red color with ferric chloride.

A series of strains representing the range of morphology in this group may be selected for characterization as possible varieties. *Sterigmatocystis glauca* Bainier was described as found on wine casks, producing white floccose masses, with sometimes a beautiful red color. This description suggests a culture received from Zellar, Missouri Botanical Garden. This form showed mycelium ranging from yellow to flesh color or rose with old fruiting areas green. In the substratum, the color produced was first orange, becoming a rich red orange in age. No. 2705 was described as *Aspergillus globosus* by Jensen, produces no floccose mycelium, changes slowly in color from white at the growing margin through shades of yellow to pale rose or dull green and varies in the amount and in the proportion of these colors in successive cultures and at successive ages of the culture, apparently as a result of differing rates of change in acidity of the medium. No. 3555.21 received from soil studies in New Jersey produced no green areas whatever, but ranged in color from white at the growing margin to a pale flesh color or pink in the center of the old colony. Cultures with the same appearance have been obtained from several separate sources, indicating the existence of a well-marked variety with these characters. Another culture represented in our collection produced little pin point colonies within a mammary gland. When the milk was withdrawn by a breast pump, interruptions of the stream would be followed by the escape of little clumps or masses which floated upon the surface of the milk. Upon examination they proved to be fruiting cultures of a member of this group bearing green heads of conidia. In laboratory culture, however,

this strain (no. 3512) grew well at 20° to 30°C., did not grow at 37°C., and failed to produce color in the substratum.

The wide range of cultural appearance is emphasized within the *A. versicolor* group by the outline presented below and by the number of described species which present the general characters of the group.

The great range of cultural appearance in the *A. versicolor* group may be illustrated by the following abstract citing a very few of the numerous cultures studied.

a. Colonies showing some shade of green in conidial areas.....b
aa. Colonies not showing green at any age.....e
b. Surface growth (in growing colonies) a felt of interwoven hyphae (floccose)..c
bb. Surface growth at first conidiophores with few or inconspicuous sterile areas (velvety).....d
c. Well marked felts or floccosity, with stalks borne only on aerial hyphae and with small and more or less scattered heads.
1. Central areas green, reverse pale 4202.3c
4235III3
4658.61/4
2. Central area pale green, reverse orange..... 4658.15/5
3. Central area pale green or greenish, reverse in carmine to purplish..... 4658.78/3
4202.9c
4700ac7
cc. Felted in center and when young, becoming almost velvety at margin later.
1. Reverse of colonies pale, almost colorless. 4291.14, 4138g31, 4658.123/2
2. Reverse orange..... 4063.B7
3. Reverse reddish orange..... 4291.15, 4658.38/8, 2706
4. Reverse purplish in age..... 2735, 4658.37/13
d. Colonies with surface growth from the first predominantly consisting of conidia bearing stalks rising directly from the substratum.
1. Colony green, reverse uncolored or nearly so.....3512
Colony green, reverse yellow, orange, in some strains progressively orange red, to red, purple red or almost black.....Many strains
2. Central area green, with marginal zone olive buff, to tan
2705 *A. globosus* Jensen
e. Colonies entirely yellowish cream, buff, to flesh color or even almost rosy; in reverse orange to deep red colors..... 3555.21
4063c
4235.I4

Possibly.....*S. spuria* Schroeter
Possibly.....*S. carnea* Van Tieghem
Possibly.....*A. koningi* Oudemans

Species in the A. versicolor group reported only as pathogens: (1) From abcess of hand in Tunis, S. tunetana Langeron, (2) From skin lesions "caraté;" South America, A. pictor Blanchard

S. tunetana Langeron. Bull. Soc. Path. Exot. 17: 345–347, with text figure. 1924; probable syn. *A. versicolor* var.?

Colonies blue-green Code de Couleurs, nos. 368, 390, 395; in age ochraceous with areas or spots finally brown (fulvus 32 in Saccardo Chromotoxia, or orange 113 in Code de Couleurs); mycelium becoming red in age; optimum growth at 25° to 30°C., slow growth at 37°C. (not a thermophile). Conidiophore simple, up to 200μ by 2.5 to 4μ; vesicle 5 to 10μ, averaging 7 to 8μ in diameter, hemispherical at apex, somewhat narrowed as inverted truncated cone toward the base passing gradually into the stalk. Sterigmata in 2 series; primary, short oblong, in form of a reversed truncated cone about 6 to 8μ by 2.5 to 3.5μ; secondary, with beak-like apex more or less elongated, grouped in 2's or 3's, 7 to 8.5μ by 3 to 3.5μ; conidia spherical, finely roughened 3 to 3.5μ in diameter, in long chains, green by transmitted light, blue-green in mass. Neither sclerotia nor perithecia found.

Culture: Langeron.

This was isolated[1] from an ulcer on the hand, but failed to produce lesions in animal experiments. The description clearly allies it with the *A. versicolor* group. Langeron finds minor differences from Mirsky's description, but when hundreds of strains have been seen the possibility of giving a specific description to such a form with any hope of identification by others becomes remote.

Species probably belonging to the A. versicolor group but doubtfully recognizable, without such a restudy of verified materials as would correctly place each of them with reference to others described in essentially identical terms

S. glauca Bainier. Bul. Soc. Bot. France 27: 29–30, pl. I, fig. 3. 1880; also Bul. Soc. Bot. France 28: 77. 1881.

This form has only been recognized since Bainier described it in 1880 in his note in 1881, that colonies on wine casks were associated with a beautiful red color which suggests that he might have had *A. versicolor*.

Colonies on extract of henbane, dregs of wine and on corks, appeared as heavy felts at first white then emerald green; heads 33.6μ diameter;

[1] Blanc, G., and Caillon, L. Sur un mycose aspergillaire observé en Tunisie. Bul. Soc. Path. Exot. 17: 343–345, 1 fig. 1924.

sterigmata primary and secondary series 10μ in long axis; conidia at first elliptical then globose, 4.2μ, smooth.

Culture: By Bainier.

S. polychroma Ferraris, Fl. Ital. Crypt. Hyph., p. 640; *A. versicolor* see Tiraboschi, Ann. di. Botanica [Rome] 7: 9. 1908.

This species was proposed by Ferraris for a strain of *A. versicolor* with somewhat smaller heads and smaller secondary sterigmata. The colonies under observation showed flesh-colored, rosy, yellow, gray, and green areas, but it is doubtful if such a strain could ever be identified a second time by the descriptive terms used, within a group in which so many strains differing only in minor details are constantly encountered.

This species was obtained from the air in the home of a pellagrous person, but no connection with the disease is shown by the report.

A. flavo-viridescens Hanzawa. Jour. Coll. Agr. Tohoku Imp. Univ. Sapporo 4: 232–3, pl. 21, figs. 1–4. 1911.

Culture no. 4291.10 received from Hanzawa under this name, hence presumably acceptable as correctly representing the species is a member of the *A. versicolor* group. This strain produces colonies at first white, then showing much yellow mycelium, later green colors with the development of conidia and finally goes over into brown; stalks 500μ long; vesicles 8.4 to 24μ in diameter, fertile in upper portions only; sterigmata primary 4.8 by 4μ, secondary 8 to 12μ in length; conidia globose, delicately spinulose 3.2 to 3.5μ. All the measurements are fairly typical for the group rather than a basis for separation of this strain.

Culture and biochemical study: Hanzawa.

S. ambari Beauregard. (Beauregard, H. Les cryptogames de l'ambre gris. Ann. de Micrographie 10: 255–278, pl. 1. 1898. Cited as *S. ambaris* without description, Compt. Rend. Hebdom. des Séances de la Soc. de Biol. 10 sèr., 5: 590–2. 1898.)

Colonies in gray-green or yellow-green colors with areas of yellow, salmon, or rose; in reverse yellow shades through orange to brownish red; medium usually colored as the reverse of the colony; stalks variable in length, up to 10μ in diameter at the vesicle; sterigmata, primary 7μ, secondary 7μ in length; conidia slightly echinulate, globose, 3μ in diameter.

Culture: Beauregard, authentic material not seen: *A. versicolor* group.

A. globosus Jensen. N. Y. Cornell Agr. Exp. Sta. Bul. 315, p. 482. 1912.

The strain studied by Jensen (no. 2705) in cultures commonly shows

a central yellowish-green to olive-green conidial area surrounded by a fairly broad zone of yellowish buff. The reverse and substratum become regularly yellowish orange followed by wine red. Microscopically this strain was typically. *A. versicolor*.

Culture: Jensen, Thom no. 2705.

S. bicolor, J. Ray. Variat. Champ. infer., p. 53. Cited by Saccardo, Syll. Ray described the mycelium as "albo-roseus," with hypostroma ochraceous and primary sterigmata filled with red coloring matter; conidia globose, spinulose, up to 2.5μ in diameter.

A. diversicolor. Waksman, S. A. Soil fungi and their activities. Soil Science 2: 126. 1916. The reference by Waksman to an organism under this name was a typographical error for *A. versicolor*.

S. spuria Schroeter, in Cohn, Kryptogamen Flora von Schlesien 3: 2 Hälfte, Lief. 1, p. 218. 1893. Syn.? *S. carnea* Van Tieghem, 1877.

Colonies at first flesh color, later dirty ocher yellow; stalk 500μ by 10μ, colorless or clear red brown; vesicle globose; sterigmata loosely standing, mostly only on upper part of vesicle, sometimes simple, again bearing secondary sterigmata; conidia globose 3 to 4μ, membrane clear reddish or yellowish, smooth, no perithecia found; from the air Breslau.

This form has not been recognized by us; it is tentatively placed in three positions in the Key from items in the description which suggest *A. versicolor* in certain of its forms and *A. flavipes* in others.

S. carnea Van Tieghem. Sur le développment de quelques Ascomycetes, Bull. Soc. Bot. France 24: 103. 1877; Saccardo, Sylloge 4: 74; and Wehmer, Monogr., p. 127.

The conidia are given as flesh color and the stalks relatively large Colonies with flesh colored heads occur in the *A. versicolor* group, but we have not seen them in nuts of Bertholletia. *A. wentii* and *A. tamarii* have been isolated from "Brazil" nuts, however, and both of these forms at times shade into colors which are close to carneus.

Culture: None; to be discarded.

Heads radiate or globose, blue green

Conidia delicately spinulose.....*A. sydowi* (Bainier and Sartory)
Conidia smooth.....*A. tiraboschii* Carbone
Possibly related.....*S. glauca* Bainier

A. sydowi (Bainier and Sartory).

S. sydowi Bainier and Sartory. Ann. Mycol. 11: 25–29, pl. III. 1913; compare plate III, 4235I7 and K22.

Colonies blue green, with the bluish effect prominent, velvety, with some aerial interlacing and trailing hyphae; reverse and substratum in shades of orange to coral red, becoming very dark or almost black in some cultures (a few strains are nearly colorless below); stalks mostly arising from submerged hyphae, up to 500μ by 4 to 8μ, colorless, smooth, thick-walled; heads radiate or globose; vesicles 12 to 20μ in diameter; sterigmata radiate in 2 series, primary (Bainier) 4 to 5 by 2 to 3μ, in our cultures up to 7 by 2 to 3μ, secondary 7 to 10 by 2μ; conidia globose 2.5 to 3μ (Bainier), in our cultures to 3.5μ spinulose, reduced conidial apparatus frequent as small heads, pencillate clusters of branched sterigmata on short conidiophores or single branched sterigmata sessile on trailing hyphae. No sclerotia or perithecia found, but chlamydospores in solid substrata are reported.

Culture: Bainier and Sartory, Thom and Church, Kopeloff.[2]

Numerous strains from Europe, Asia and many places in America establish this as a cosmopolitan group, in which considerable variation in reactions and details of structure are encountered. A typical member of this series selected by us and submitted to Bainier established the correctness of our identification of the group.

A. tiraboschii Carbone. Atti d. Ist. Bot. Univ. Pavia, ser. II, vol. XIV, p. 320. The characterization "yellow-green" allies this form with *A. versicolor* rather than *A. sydowi*.

The description corresponds closely with this grouping. "Caespitosus aerugineo viridis. Hyphis sterilibus hyalinis, subcontinuis 1.5μ latis; fertilibus hyalinis, simplicibus, parce septatis, basi leviter attenuatis, 4 by 140μ; capitulis flavo-virentibus, sphaeroidalibus, 140 to 230μ latis, 125–185 longis: vesicula hyalina pyriforme, 13μ lata; Sterigmatibus primariis hyalinis, cylindraceis, parum minus latis quam longis (5μ), vesiculam totam tegentibus, sterigmata secundaria ternata, hyalina, acuta, 1.5μ lata, 6.5 longa gerentibus; conidiis catenulatis, globosis, flavo-virentibus, laevibus, 3μ diam. Habitat in botulis, (Salame crudo) Papiae."

The colony characterization of aerugineous green is contradicted by the color given for the heads as yellow-green. Unless reisolated and studied recognition must remain doubtful since a request for cultures failed.

[2] Kopeloff, N. and L. The deterioration of cane sugar by fungi. Louisiana Agr. Exp. Sta. Bul. 166. 1919; also in Factors determining the keeping quality of cane sugar. Ibid., no. 170. 1920.

SECTION VIII

A. TERREUS, A. USTUS AND CONNECTING FORMS

Stalks smooth.
Conidial heads never green.
Conidial heads avellaneous or brown (or black?); conidia not roughened with bars and tubercles of color.
Sterigmata in 1 series, but with the aspect of *A. fumigatus* and *A. nidulans*.
1. Conidial chains in a column; from soil.....*A. calyptratus* Oudemans
2. Conidial chains radiate.
a. Pathogenic.....*A. gratioti* Sartory
b. Not pathogenic; fawn colored; conidia 2μ....*A. cervinus* Massee
Sterigmata in 2 series.
1. Colonies avellaneous; conidia in columnar masses 2–3μ...*A. terreus*
Probable synonym.....*A. fuscus* Amons
2. Conidial chains radiate.
a. Conidia 3 to 4μ rough.....*A. ustus*
b. Conidia up to 5μ rough.....*A. insuetus*

A. calyptratus Oudemans. Arch. Néerl. (II) 7: 283, pl. 13. 1902. The data given are: Stalks 200 to 300μ long; vesicles 20 to 22μ in diameter; sterigmata in one series about 6μ long; conidia globose, gray, 2.3μ in diameter; on oak leaves; Bussum, Holland. The conidia are described as forming a black column which is, however, figured as brown. Whether there is a black or brown species with characters so closely resembling *A. fumigatus* as this, we have not determined; certain strains of *A. fumigatus* might easily have furnished this description.

Culture: Koning, Oudemans.

Goddard (Goddard, H. N. Bot. Gaz. 56: 267, fig. 8B. 1913) uses this name for cultures which are clearly *A. fumigatus* by description, by figures and by constant occurrence in soil culture work.

A. calyptratus Oudemans var. *italicus* Ferraris. In Ferraris, T., and Massa, C., Micromiceti nuovi o rari per la Flora Micologica Italiana, Ann. Mycol. 10: 294. 1912. This is described as differing from type in its longer chains of conidia (420 to 450μ). It was found upon the surface of *Tuber melanosporus* decaying in an alcoholic solution. The ground for separation is hardly sufficient even if this culture were assigned correctly to the species.

Culture: Presumably by Ferraris. This organism has not been seen by us.

A. gratioti Sartory. (Sartory, A. Sur un champignon nouveau du genre Aspergillus isolé dans un cas d'onychomycose. Compt. Rend. Acad. Sci. [Paris] 170: 523–524. 1920); also in Champignons parasites de l'homme et des animaux, p. 578, 579. 1922, but without further data.

Colonies on Raulin's solution agar gray with a wet appearance, then brown and finally almost black, becoming an uneven blisterlike wrinkled mass with white margin; conidial areas brown running out as fine powdery brown lines into the white area; hyphae septate, 0.6 to 1.5μ in diameter richly branched; stalks short, delicate 4 to 5μ in diameter enlarging gradually upward to vesicles 8 to 20μ in diameter and nearly globose; sterigmata in 1 series about 6μ in length, crowded over the whole surface of the vesicle; conidia globose brown 3 to 3.5μ, separating readily. Sclerotia and perithecia not found. Chlamydospores 20 to 40μ in diameter seen imbedded in tissue of skin and nails.

Culture: Sartory.

This species was isolated from the nails and surrounding skin of a Chinese traveling in France. Colonies grew upon Raulin's solution with various sugars, upon carrot, potato, potato with glycerine, Sabouraud, bouillon gelatin and milk, but not upon coagulated beef serum, egg albumen, or acid potato. It did not attack lactose, galactose or saccharose. The species has been reported but once. No reports of pathogenesis to animals were given. The description given suggests the characterization of *A. calyptratus* as given by Oudemans.

A. cervinus Massee. Kew Misc. Bul. 4: 158. 1914. Described from African soil, near Khartoum; not reported elsewhere. Colonies forming an effused fawn colored stratum on culture media; stalks, scattered, or sparse, 80 to 350 by 8 to 10μ; sterigmata 7 to 8 by 3μ, in one series; conidia globose, smooth, 2μ.

Culture: Massee; no. 3522.36 from Porto Rico soil was close to this form, but proved to be difficult to maintain in culture hence was soon lost.

A. terreus Thom, in Turesson, Göte, Svensk Botanisk Tidskrift 10: 5. 1916, without description; diagnosis Thom, C., and Church, Margaret B., *Aspergillus fumigatus, A. nidulans, A. terreus* n.sp. and their allies, Amer. Jour. Bot. 5: 85–6. 1918.

Colonies upon Czapek's solution agar from tints of pinkish cinnamon through cinnamon (at times near avellaneus of Saccardo's Chromotaxia)

to deeper brown shades in age (see Ridgway, pl. XXIX, 15″). Klincksieck and Valette, nos. 103D, 112, 113, 108); spreading, velvety or in some strains developing definite floccosity or anastomosing ropes of aerial hyphae; reverse and agar from pale or bright yellow to fairly deep browns. Odor, none in some strains, at least transiently present in others, or developing with the addition of high percentages of cane sugar. Conidiophores 5 to 8μ in diameter, 50 to 150μ or even 250μ in length, more or less flexuous, with walls smooth colorless or nearly so up to 1μ thick, septate or unseptate, with apex enlarged to form a vesicle commonly 12 to 18μ, occasionally up to 25μ in diameter, bearing

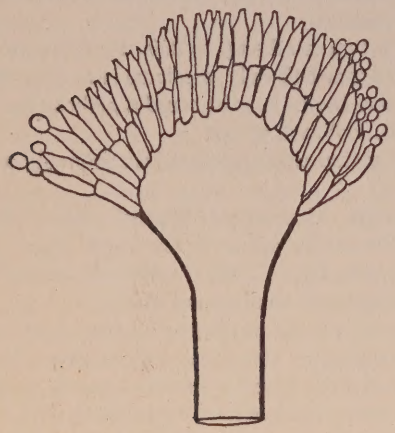

FIG. 14. A. TERREUS

Shows a hemispherical type of head, with both primary and secondary sterigmata.

sterigmata usually in two series upon its domelike upper surface (fig. 14); primary sterigmata 2 to 2.5μ by 7 to 9μ, secondary 2 to 2.5μ by 5 to 7μ closely packed; heads becoming solid columnar masses up to 500μ long by 50μ in diameter; conidia slightly elliptical to globose, 2.2 to 2.5μ or even to 3μ in diameter, smooth, in long, parallel, adherent chains. Perithecia not found. Grows at 37°C. Liquefies gelatin.

Habitat: Common in soil and in decaying vegetable matter, throughout the United States.

Search of the early literature shows a description and figure closely resembling *A. terreus* as *Rhodocephalus aureus* Corda, Icones 3: 12,

taf. II, fig. 33. 1839, but positive identification is not possible. As indicated in previous discussion, *A. terreus* differs from *A. nidulans* in failing to produce asci and in entire lack of green color, with general increase in the intensity of its avellaneous coloring element.

Culture: Thom and Church. This organism is common, especially in soil in America but has not been received by us from European sources.

A. fuscus Amons. (Amons, W. J. Th. Bijdrage tot de Kennis van de Flora van Achteruitgaande Suiker. Archief voor de Suikerindustrie in Nederlandsch-Indië Jaarg. 29, Deel 1, January–June, 1921, pp. 8, 9, 10; probably a synonym of *A. terreus* Thom; not *A. fuscus* of Bonorden, of Bainier or of Schiemann.)

Colonies light brown; stalks up to 200μ by 6μ smooth; vesicles 16 to 18μ fertile on the upper half only; sterigmata in 2 series, primary about 8μ, secondary about 10μ in length; conidia globose 2μ.

Culture: Amons; not seen by us.

From this description this organism was probably a close relative of *A. terreus*.

S. hortai Langeron. (Langeron, Maurice. Sur un champignon d'une otomycose brésilienne: Sterigmatocystis hortai n.sp. Bull. Soc. Path. Exotique 15: 383–384, fig. 1. 1922.) Syn. *A. terreus* Thom probably; or *A. nidulans* with green color suppressed.

Colonies clear ochraceous; stalks figured as short, smooth, more or less sinuous, enlarging to domelike vesicles about twice the average diameter of the stalks; vesicles described as 5 to 10μ in diameter; sterigmata in 2 series, each about 5μ in length by 1.5μ in diameter, secondary sterigmata in 2's or 3's; conidia in long chains, smooth about 2.5μ in diameter; the conidial mass is figured as a dense mass spreading somewhat at the apex but practically a column.

This organism formed a dry membranous mass (mycelium) in the ear of a Brazilian. The description and figures of Langeron resemble *A. nidulans*, except for the entire absence of green color in the colonies. Although in some strains of *A. nidulans* and under some cultural conditions, this green color is largely suppressed, it is too conspicuous when present to have escaped notice in repeated culture, hence the organism was probably a strain or variety of *A. terreus*. *A. terreus* is widely distributed and varies considerably in intensity of color and in colony appearance.

A. ustus (Bainier); Syn. *S. usta* Bainier. Bull. Soc. Bot. France 28: 78. 1881. The organism described by Bainier remained long unrecog-

nized by us on account of the total inadequacy of the description given. In the course of years a collection of strains from widely separate sources lead to the following group characterization to which the name **A. ustus** (Bainier) is attached, because of culture no. 4640.488 received in the Bainier Collection as *S. usta* (compare plate III, 3556).

Colonies more or less felted, floccose, with fine hyphae, from white through shades of gray, olive gray, yellow, yellow brown toward fuscous, with often a greenish cast, but no true green color, in old cultures purplish vinaceous at times: reverse through shades of yellow, orange and brown; stalks when rising from submerged hyphae up to 1000μ, mostly branches of aerial hyphae up to 500μ by 5 to 10μ, few septate, sinuous, with walls rather thin, smooth, usually partly colored some shade of brown; vesicles 10 to 20μ diameter; heads hemispherical to almost columnar; sterigmata colorless, semi-radiate, loosely arranged into 2 series, primary 5 to 8 by 3μ, secondary 7 to 9 by 2 to 2.5μ; conidia globose about 3.6μ (3.5 to 4μ) spinulose, or with fine faint bars of rosy, reddish yellow or vinaceous color, with chains forming fairly compact columns in old cultures. Some strains show sterile clusters of thick-walled helicoid cells, comparable to the "Hülle" cells of *A. nidulans*, but perithecia have not been found.

Culture: No. 4640.488 in the Bainier Collection as received under this name belongs to a group common in America and otherwise not described. Another culture 4202.17T was received from Professor Thaxter under the manuscript name of *A. helicophorus*.

In spite of the accumulation of a series of cultures from many sources, we have been unable to identify this series with any other name, except to believe that Aspergillopsis of Sopp covers this group.

A. insuetus (Bainier). *S. insueta* Bainier, Bull. Soc. Mycol. France 24: 85–87, pl. VIII, figs. 1–13. 1908; also discussed by Sartory, Étude biologique du "S. insueta" sur les divers milieux, ibid.: 221–229.

As described: Colonies entirely fuligineous, with more or less abundant branching aerial mycelium bearing the conidiophores as branches usually short; vesicles 11 to 16μ in diameter; sterigmata either regularly produced as primary sterigmata 5 by 8μ, with very short secondary sterigmata or with proliferation of primary sterigmata to form a second or more series before the conidia-bearing series is reached; conidia globose, rough, up to 5μ in diameter, fuligineous, with the color mostly aggregated into the echinulations of the cell-wall, and even forming bars and tubercles at times.

Culture: By Sartory, showing optimum temperature 34° to 35°C.,

cessation of growth at 38° to 39°C., growth on all common media, and no pathogenicity to laboratory animals. Two cultures, no. 4658.245 from South Africa and no. 4303.65 from Louisiana, have reproduced the morphology of Sartory's species closely enough to warrant identification. In these cultures, colonies spread rapidly in the substratum, became yellow then brown to black in reverse, with surface growth floccose, almost black in the center shading through gray to white sterile floccose marginal areas while growing; central areas show scattered groups of thick-walled variously coiled or curved cells resembling the "Hülle" cells described by Eidam for *A. nidulans* and found in the groups related; the stalks were up to 300μ by 4 to 8μ, with walls brown, smooth about 1μ in thickness; heads washed free from conidia 35 to 40μ in diameter at the broadest place; sterigmata more or less radiating from the upper two-thirds of the vesicles which were about 15μ in diameter; sterigmata were produced irregularly, partly as primary and secondary series, partly as proliferations almost as short septate hyphae; conidia globose with inner and outer layers of wall distinguishable and the inner wall spinulose or rough, with "connective" prominent, 3.5 to 5μ in diameter. This species suggests Sopp's[1] genus Aspergillopsis, but does not comply with the detailed description of his *A. fumosus*.

[1] Sopp, O. Johan-Olsen. Videnskapsselskapets Skr. 1. Mat.-Naturv. Kl., no. 11, pp. 201–202, taf. XX, fig. 149. 1912.

SECTION IX

The White-Spored Aspergilli: A. flavipes, A. candidus and Connecting Species toward the Black Aspergilli

Conidial heads white (some forms shading toward avellaneous or yellowish in age)

Chains of conidia mostly in columnar masses; stalk walls yellow; with or without sterile aerial masses of hyphae or submerged sclerotia-like masses or crusts.....*A. flavipes* group

Chains of conidia radiate (heads mostly globose); stalk walls mostly uncolored or only partly colored near the vesicle. Perithecia not found.....*A. candidus* group

Perithecia reported.....*A. albidus* Eichelbaum

Heads some shade of yellow

Heads few, yellow; sclerotia abundant, black..*A. alliaceus* Thom and Church

Heads ocher yellow; morphology of *A. candidus*.....*S. ochracea* Bainier

Heads pale yellow; stalks fasciculate.....*S. veneta* Massalongo

Heads pale yellow; conidia verrucose 5 to 6μ.....*S. corolligena* Massee

Heads pale cinnamon; conidia smooth.....*A. cinnamomeus* Schiemann

A. FLAVIPES GROUP

A. flavipes group. A whole series of forms show characters essentially resembling *S. flavipes* of Bainier and Sartory. Members of this series have been collected by us from many substrata; some were received from the sugar products of Kopeloff; some have been received from the soil studies of Waksman in New Jersey, and Abbott in Iowa. Another from the Bainier collection and one from Pribram.

The range of appearance and reactions follows (compare plate III, 114): Submerged mycelium from persistently colorless to yellow, orange, orange-brown and in some strains showing areas deep brown or almost black and developing irregularly globose or tubercular submerged or protruding sclerotia or others prostrate stromatic (?) masses. Aerial mycelium more or less abundant colorless to yellowish, in some strains forming more or less abundant, closely woven, yellow masses containing many helicoid, to horse-shoe shaped thick-walled cells resembling the "Hülle" surrounding the perithecia of *A. nidulans* but remaining sterile as far as studied; heads mostly becoming columnar or calyptriform masses commonly persistently white but with some strains in pale avellaneous shades to deep avellaneous as in *A. terreus;* stalks from

300 to 500μ by 4 to 5μ, or up to 2 to 3 mm. in length and 8 to 10μ in diameter in some strains and under some conditions of culture, yellow under the microscope, in mass some shade of buff to vinaceous buff, with color mostly localized in the outer layers of cell-wall and occasionally as disklike concretions on the surface of walls otherwise smooth; vesicles subglobose to elliptical up to 20 by 30μ in the largest forms, usually with diameter twice that of the stalk in smaller forms; sterigmata in two series, colorless or nearly so, closely packed over the apex of the vesicle in small heads, and covering the vesicle in large heads, primary sterigmata about 2 to 3 by 4 to 7 or 8μ, secondary sterigmata 1.5 to 2 by 5 to 8μ; conidia 2 to 3μ, smooth, subglobose, colorless or nearly so in mounts, in chains aggregated to form columns as seen with the handlens in old cultures.

The various strains in this group differ in their gross characters from almost velvety colonies with abundant stalks of uniform length and nearly white until very old, to colonies with abundant cottony aerial mycelium in shades of gray, buff or vinaceous buff with sparse development of long stalked white calyptrate heads, or to more closely felted forms with brown sclerotia or brown crusts upon or just above the substratum, and to colonies with more or less numerous yellow aggregations of sigmoid or helicoid cells suggesting the possibility of perithecia. In their most diverse form, several species might be easily described, but when large numbers of strains are brought together and cultivated over a considerable range of substrata the continuity of the group as a natural and related series of strains becomes apparent. Further study of this whole group may lead to separation upon lines accounting for certain published descriptions not at present identifiable.

A. flavipes (Bainier and Sartory).

S. flavipes Bainier and Sartory. Bul. Soc. Myc. France 27: 90–96, pl. III, figs. 1–6. 1911.

Cultures from the Bainier collection (4640.474 and 4640.402) show a form with the outer layer of the stalk wall yellow; stalks 300 to 400μ long, by 3 to 4μ in diameter; vesicle about 7μ in diameter; sterigmata, primary about 5μ in long axis, secondary about 7μ long; conidia colorless thin-walled about 3μ.

A group of strains have been found in America which belong in a group with this culture, hence have been designated the *A. flavipes* group. Whether *A. rehmii* Zukal belonged with this species has not been determinable since thus far none of the masses of hyphae suggestive of perithecial development have proved fertile.

A. CANDIDUS GROUP

A. candidus Link, Obs. p. 16. 1809. "*A. candidus*, caespitibus laxis, floccis albis erectis, capitulis concoloribus. Aetate color non mutatur. In fungis putridis." As indicated by Van Tieghem in his discussion of *S. alba*, there are a whole series of white Aspergilli which can be grouped together and may be assumed to represent Link's *A. candidus*.

Group characters (compare plate II, 106): A broad characterization of this group follows: Colonies (on Czapek's solution agar especially) white persistently, or becoming cream or yellowish cream in age; sclerotia produced in occasional strains and under special conditions (purple sclerotia in no. 4781) but not common; surface growth usually stalks and heads with scanty sterile mycelium, or anastomosing ropes of hyphae bearing short fertile stalks (in 4658.54/3 suggested as *A. veneta*) in an occasional strain; stalks varying with the strain, in short or dwarf races less than 500μ long to strains with stalks 500 to 1000μ or longer, varying from 5μ in diameter in dwarf forms to 10 to 20μ in long stalked forms, with walls thick, smooth, colorless or slightly yellowed near the vesicle in certain strains in age; heads white, globose, radiate, varying in the same culture from large globose masses 200 to 300μ in diameter to small heads less than 100μ in diameter, globose or more or less elongated, with incomplete development of sterigmatic surface; vesicles typically globose up to 50μ in very large heads, and fertile over the whole surface; sterigmata typically in 2 series, usually colorless, primary varying greatly in the different strains and under different conditions and ages in the same strain, from 5μ in length in small heads to 10, 15 or 20μ under other conditions, secondary 5 to 8 by 2 to 2.5 or 3μ; conidia colorless, globose or subglobose, thin-walled, 2.5 to 3.5 or occasionally 4μ, smooth.

The white Aspergilli grow more slowly than many other forms, hence are seen commonly in fairly dry substrata, such as slowly spoiling cereals, and in other situations in which the more rapidly growing species are more or less suppressed. Upon spoiling seeds, the white Aspergilli show as groups or tufts of long stalks with shining white heads. Comparative study is needed to furnish lines of separation among these forms, which are very unsatisfactorily described thus far.

A. okazakii Okazaki. (Okazaki, K. Eine neue Aspergillus-Art und ihre praktische Anwendung. Centralb. f. Bakt., etc., 2 Abt., 19, pp. 481–484, taf. I. 1907; see also Centralb. f. Bakt., etc., 2 abt., 42, p. 225. 1914). This is cited by Saccardo as *S. okazakii* Saito in Sylloge 22:

1260. 1913, giving the same reference, but Saito seems only to have been one of four workers who suggested to Okazaki the name under which he published his description and figures.

Colonies white to sulphur yellow; stalks hyaline, straight or sinuate, smooth or asperulate, 200 to 500 by 8 to 12μ figured as undulate, especially toward the base, with walls 2 to 3μ thick; heads 80 to 100μ in diameter; vesicles 12 to 40μ diam.; sterigmata primary 15 to 20 by 6 to 8μ, secondary 8 to 14 by 2.5 to 4μ; conidia globose, hyaline, 2.5 to 5.4μ smooth, with connective. Culture and biochemical study: By Okazaki as the basis of an enzym preparation "Digestin" and compared by Okazaki with *A. albus* and *A candidus*. Kita (Kita, G. Centralb. f. Bakt. etc., 2 Abt. 37: 440–452. 1913.) discussed the biochemic activities of *A. okazakii* (*S. okazakii*) in connection with another white form regarded by him as a new species but not named. The morphological characters given are hardly adequate to separate these forms. *A. okazakii* was the basis of Japanese patent 11461; culture no. 4337 from Takahashi varies from this description in that the stalks of Hanzawa's culture, while not definitely pitted, have markings occasionally toward the base which may justify Okazaki's term asperulate. Further study of these white forms is desirable.

Species difficult or impossible to separate from A. candidus by the information available, hence probably to be discarded

S. alba Bainier. Bul. Soc. Bot. France 27: 30. 1880; probably *S. alba* Van Tieghem, Bul. Soc. Bot. France 24: 103, 1877, nomen nudum.

Described from oatmeal, oats, and the carton. This is a frequent substratum for the common forms of white Aspergillus. Heads 35 to 45μ in diameter, washed free from conidia (? CT); stalk up to 8.4μ in diameter; sterigmata, primary up to 16.8μ, secondary up to 12.6μ long; conidia about 3.2μ.

Culture: Bainier, but without complete reports.

Van Tieghem (1867) gives the name without description, but reports that it is one of several white Aspergilli known to him and that it is probably *A. dubius* Corda as discussed by Cooke and Berkeley (Handbook of British Fungi 2: 588. 1871).

S. candida Sacc. Michelia 1: 91. 1877; see also F. italici t. 80.

Saccardo here described a white Aspergillus of decaying insect larvae in terms fairly general except the measurements of stalks 14 to 15μ in diameter, and conidia 2.5μ in diameter and hyaline aggregated into a

head 100 to 120μ in diameter. Schröter (in Cohn, Krypt. Sch. 3: 218. 1893) expands the range of measurements included under the name until it is broad enough to cover the common forms encountered in culture and described here as *A. candidus*, although Saccardo did not consider his species a synonym of *A. candidus* Link.

A. albus Wilhelm, Beitr. z. Kenntn. d. Pilzgattung Aspergillus. Inaug. Diss. Strassburg, p. 69. 1877. A white Aspergillus, for which the author cites as probable synonyms, *A. niveus* Micheli, *A. candidus* Link, *A. albus* Haller, *Monilia alba* Gmelin, *Monilia candida* Persoon, *A. dubius* Corda; Syn. *S. alba* Sacc.; Syll. 22: 1260. 1913.

Diagnostic information: Stalks, colorless, "vitreous," smooth, about 500μ long by 7μ; heads white (candidus); vesicle up to 30μ; sterigmata in two series, measurements not given; conidia globose, smooth, colorless, 2.7 to 3.5μ.

Culture: By Wilhelm; not separable from other members of the *A. candidus* group by the description given.

S. candidula Bainier, in Saccardo Syll. 4: 73. 1886; syn. *S. candida* Bainier, Bul. Soc. Bot. France 27: 30. 1880.

Described as a very common white mold on leaves; stalk 6.3μ in diameter; "capitule" (? = vesicle) 16.8μ in diameter; sterigmata, primary 4.2μ, secondary 10.5μ long; conidia white, smooth, globose, 3.1μ in diameter.

Culture: Not reported; apparently described from herbarium material.

Saccardo evidently changed the name of Bainier's species on account of his own prior use of *S. candida*, but failed to indicate his own responsibility for the change.

S. coronata Van Tieghem. Bul. Soc. Bot. France 24: 103. 1877.

Described as a form with white heads up to 1 mm. in diameter in which secondary sterigmata are borne as crowns of 10 to 12μ upon the apices of the primary sterigmata; conidia globose 1.5 to 2μ.

Culture: Not reported. This organism has not been identified by us.

Van Tieghem designates his form as white and introduces no data to exclude it from the synonymy of *A. candidus*.

S. coronella Costantin, Mucéd. Simples, p. 34, fig. 2. 1888.

Described from dead wood: Colonies white; stalks described as 16.8μ near apex and 19.6μ in diameter at base; vesicle up to 40 to 50μ in diameter; sterigmata in 2 series on the upper part of vesicle only, and about equal in length as figured; conidia oval oblong 4.9 to 5.6 by 2.7 to 3.8μ.

Culture: None. Placed with *A. candidus* group but not identified by us.

A. dubius Corda, Icones Fungorum II, taf. XI, fig. 77. 1837. Probably not *A. dubius* of Berkeley and Broome, Ann. Nat. Hist. 7, 2 sèr., p. 100, no. 520. 1851. See *S. dubia* (Corda), Sacc. Fungi Italici, t. 902, and Sylloge 4: 72, which appears to be based upon Berkeley and Broome's material. Van Tieghem, Bul. Soc. Bot. France 24: 103. 1877, identifies the organism given by Cooke and Berkeley (Handbook of British Fungi 2: 588. 1871), as his *S. alba*. Bonorden, Handbuch allg. Myk., p. 112. 1851, suggested that this is a synonym for *A. ovalispermus* Link, but no grounds for the identity are offered.

Described from Swiss and Holland cheeses, as colonies white, small stalks, simple, straight pellucid (pitted ? but not so figured); vesicle described and figured as cut off by a cross-wall; conidia obovate, unequal in size with wall colorless but more or less yellowish cell contents. Corda designates the stalk as "pellucido," which can only be fairly interpreted as pitted or roughened as seen in the *A. flavus* group; this interpretation is further strengthened by the size and ovate form of the conidia and the simple sterigmata of Corda's figure. Corda's designation of this species as white, however, has caused its inclusion in that group by Saccardo, Berkeley, Rabenhorst and others.

Culture: None. To be dropped.

A. fimetarius Peck. N. Y. State Mus. Bot. Rept. 42 (1888): 128. 1889.

Colonies white; stalks simple, septate, slightly enlarged at apex; basidia (sterigmata) oblong or subcylindrical 12 to 15μ long, presumably in one series (if correct, not *A. candidus*); conidia globose 4 to 5μ in diameter. Found in excrement of deer in the Adirondack Mts.

Culture: None.

The species was separated by Peck from *A. candidus* on account of its larger spores and septate stalks, but has not been identified since. Although Peck did not find double sterigmata, this species may be placed with *A. candidus* as a synonym unless it is rediscovered and its true affinities more completely described. There is much variation in details of structure in the heads of this group and in the measurements observed.

A. mycobanche Link. Sp. Plant., Ed. IV. Vol. VI. pt. 1. p. 65. 1824. The description of this fungus from rotting Peziza furnishes no mor-

phological data for identification aside from placing it among the white forms.

Culture: None; not since recognized.

A. ovalispermus Link. Obs. II, p. 37. 1816, also Sp. Plant., Ed. IV, vol. VI, pt. 1, p. 66. 1824. Cited as synonym of *A. oosporus* Wallr. (1833) without evidence of identity or reason for redescription. The description gives no data which can be used to determine which of a number of oval spored forms was under observation. Link lists this form among species always white but there is no continuous use of the name to furnish clues to its identity.

Culture: None; to be dropped.

No Aspergillus could be found upon a specimen marked *A. ovalispermus* from the herbarium of Schwienitz, in the Curtis Collection.

A. oosporus Wallroth, Flora Cryptogamica Germaniae no. 1928, in Compendium Florae Germanicae 4: 296. 1833. "Hyphis robustus densis erectiusculis, sporidiis ovalibus incumbentibus albis. *A. ovalispermus* Link Obs. II: 37." No further data were given and no ground was offered for changing the name of Link's organism reported on rotten apples or for claiming identity with it.

S. pulchella Speg. Spegazzini, C. Fungi argentini. Sociedad Cientiffica Argentina Anales 10: 163. 1880.

Colonies white, delicate; stalks hyaline, 200 to 250 by 5μ; vesicles 40 to 50μ in diameter; primary sterigmata 16 to 20 by 3 to 5μ, secondary sterigmata in 3's (?) 5 by 2μ; conidia colorless, globose, smooth 5μ in diameter. In old leather kept in a moist place.

The material was not cultivated but described as found upon the leather. While the conidia were large for the *A. candidus* group, the other data point to that group relationship. Until rediscovered and cultivated the species must be considered doubtful.

A. sterigmatophorus Saccardo, Mycologicae Venetae Specimen. Atti della Societa Veneto-Trentina di Scienze Naturali 2, fasc. 2: 232. Tab. XVII, fig. 5–8. 1873; (Myc. Venetae Specimen was republished in repaged form giving this species on p. 184) syn. *S. italica* Sacc. F. italici no. 109, 1881; changed to *S. italica* Sacc. as a note only in Michelia 1: 91. 1877; Latin diagnosis of *S. italica* in Saccardo Sylloge 4: 72. 1886.

Described from decaying corn kernels (*Zea mays*): white, sparse, with stalks unbranched, 2 to 3 septate above; with vesicles globose; sterigmata described as dichotomous or trichotomously branched with

ultimate cells bearing conidial chains; conidia globose about 6μ in diameter, with connective.

The various figures and descriptions given by Saccardo all appear to be based upon a single collection of a white Aspergillus on rotten corn grains. Dichotomous or trichotomous branching of the sterigmata was the sole basis in the author's mind for separating the species. Until someone else finds a white Aspergillus with conidia about 6μ in diameter and possibly secondary sterigmata in 2's rather than in larger number, the species must be regarded as doubtful.

S. szurakiana Moesz, G., Mykologiai Közlemenyek IV, in Bot. Közlem., t. 19: 59, text fig. 10 on p. 60. 1921.

Diagnosis in Latin translated: Sterile hyphae colorless, creeping: stalks erect, unbranched 1 to 2 mm. long by 16 to 17μ in diameter, unseptate, colorless, bearing globose white heads of conidia 145 to 210μ in diameter; vesicles globose, colorless, punctate, 45 to 55μ in diameter; primary sterigmata radiating, clavate, with a constriction above the middle, 32 to 40 by 10μ; secondary sterigmata regularly in 4's or 5's cylindrical to fusiform, 7 to 10 by 3μ; conidia globose, smooth, colorless, 3 to 3.5μ in diameter in long chains.

Habitat on hemp rope, Budapest, Hungary.

This organism does not appear to have been cultivated. While the particular strain growing upon Szurak's rope may have had characters not included in the description, various strains of the *A. candidus* group might easily have furnished the material for this description.

A. albidus Eichelb. (Eichelbaum, F. Verhandlungen d. Naturw. Ver. Hamburg 3 Folge XIV: 35, 1906; syn. *E. albidum* Sacc. Syll. 22: 1254. 1913. This ascogenous form is described as bearing the conidial apparatus of *A. candidus* but with perithecia so described as to suggest the *A. glaucus* group.

As described: Perithecia shining white on white mycelium with the same measurements as *Eurotium herbariorum;* asci comparatively persistent, nearly globose (rundlich) 10μ in diameter; ascospores smooth, white, hyaline, roundish ovate, 3 by 4μ (markings, if any, not mentioned). Conidial apparatus is *A. candidus* Link. Found with *A. herbariorum* on badly dried tobacco.

Inoculation of tobacco with a member of the *A. glaucus* group producing small ascospores did not affect the colors produced by that species. Until Eichelbaum's organism is rediscovered and cultivated it must be arbitrarily placed in that part of the group of white Aspergilli whose identity is doubtful.

Heads more or less yellow

A. alliaceus n. sp. Discussed without name as no. 4660, Walker, J. C., and Lindegren, C. C., in Further studies on the relation of onion scale pigmentation to disease resistance. Jour. Agr. Res. (1924) 29: 507–514. 1925, and by Walker, J. C., Lindegren, C. C., and Bachmann, F. M., in Further studies on the toxicity of juice extracted from succulent onion scales, Jour. Agr. Res. 30: 175–187. 1925.

Colonies on Czapek's solution agar with white floccose mycelium spreading rapidly over the surface of the substratum, and quickly producing abundant sclerotia, at first white, later becoming black without yellow or orange colors, ovate to elliptical up to 500 to 700μ in horizontal diameter, up to 1000μ or more in vertical axis, with a depression or pore (?) at the apex; ascigerous forms not found; producing few and scattered stalks with heads up to 200μ in diameter, yellow or becoming ocher to brown in age; stalks up to 1500μ by up to 15μ, with walls colorless, smooth 1.5μ in thickness breaking with rough or ragged edges; vesicles up to 40 to 50μ in diameter with wall 1.8 to 2μ in thickness and showing prominent pores at bases of sterigmata; sterigmata primary 7 to 12 by 2 to 4μ, secondary 7 to 8 by 2μ colorless; conidia faintly yellowish, elliptical to globose 2.5 by 3μ, to 3μ in diameter.

This organism was isolated by Walker and his co-workers in the study of rots of onions and is regarded by them as more destructive than *A. niger*. Another strain (no. 4656) identical with this in morphology was obtained by us from a dead blister-beetle (*Macrobasis albida*) furnished by the Pharmacognosy Laboratory of the Bureau of Chemistry. Many experiments to induce the development of an ascogenous phase in these sclerotia whose appearance is so suggestive of perithecia have thus far failed.

S. ochracea Bainier. Bul. Soc. Bot. France 28: 78. 1881.

Colonies long-stalked, ocher yellow, as grown on solution of tartrate and salicylate of sodium; stalk 10.5μ in diameter, uncolored, smooth; heads (? vesicles) globose 52.5μ in diameter; sterigmata, primary 10.5μ, secondary 6.3μ in length; conidia globose, smooth 3.2μ.

Bainier's description of ocher yellow heads on smooth colorless stalks puts his form nearer the *A. candidus* group rather than to *A. ochraceus* in the sense of Wilhelm. Some strains of this group become quite yellow in age.

Culture: Bainier; not identified since publication; to be dropped.

S. veneta Massalongo. Bull. Soc. Bot. Ital., no. 7–8, p. 159. 1900.

This form is described as forming yellowish hemispherical colonies upon rotten twigs; the fertile hyphae are described as "fasciculate" (form coremia?). Werkenthin (Fungous flora of Texas soils, Phytopathology 6: 247–249. 1916) identified certain cultures from Texas soil as *A. venetus* on account of fascicles or ropes of aerial hyphae. Cultures of these forms received from Werkenthin show that he had strains of *A. terreus*.

The following measurements are given by Massalongo: Stalks 5 to 7μ in diameter; vesicle subglobose, obovate 20 to 26 by 14 to 20μ; sterigmata, primary 4 to 6 by 2 to 3μ, secondary 6 to 8 by 2μ; conidia globose 2 to 3μ scarcely yellow.

Culture: Apparently none.

Massalongo separated his organism from *A. sulfureus* on account of the pale yellowish color and the elliptical vesicles. While not positively identifiable as this species, no. 4658.54/3 from South Africa very nearly satifies this description.

S. corolligena Massee. Bul. Royal Bot. Gard. Kew no. 1, p. 5. 1910. Described from corollas of Impatiens from Manipur, India.

Heads sulphur yellow; vesicle globose; sterigmata in two series; conidia globose, yellowish (flavida), verrucose, 5 to 6μ. Massee suggests it may be *S. sulphurea* Fres., but separates it on account of the globose warted conidia.

Culture: None.

Massee's description gives this organism the morphology of the *A. niger* group with yellow heads and conidia larger than the typical black forms.

A. cinnamomeus Schiem. (Schiemann, Elisabeth. Mutationen bei Aspergillus niger Van Tieghem. Ztschr. Induktive Abstam. u. Vererbungslehre, Bd. 8, Heft 1/2, pp. 1–35, 16 fig., 2 pl. (1 col.). 1912.) Differentiated by color and smoothness of spores from *A. niger* Van Tieghem but maintains these distinctions uniformly. Obtained as a mutant from *A. niger* in culture by Schiemann.

Culture: Schiemann; our no. 3534b.

SECTION X

The Black Aspergilli, A. niger and Allies

Species regarded as identifiable either as individual strains or as representing groups of strains with fairly consistent morphology.

I. Color almost suppressed.
  A. cinnamomeus Schiemann, pale cinnamon color, otherwise *A. niger* (not black; see previous group).

II. Color diffused, not aggregated in tubercles or bars.
  A. schiemanni (Schiemann) Thom, fuscous colony, otherwise *A. niger*.

III. Color purple-brown to black.
  A. Sterigmata in 1 series.
    A. nanus Mont., conidia 3μ; sterigmata up to 15μ long.
    A. luchuensis Inui, conidia 4 to 5μ; sterigmata 7 to 9μ.
  AA. Sterigmata in 2 series.
    B. Conidia elliptical.
      A. violaceo-fuscus Gasp., conidia elliptical 3.3 to 5 by 5 to 6.5μ.
    BB. Conidia globose or subglobose.
      1. *A. luteo-niger* (Lutz) Thom and Church, conidia about 5μ with color bars breaking up in mounts to leave walls smooth.
      2. *A. niger* Van Tieghem, conidia 2.5 to 4μ, primary sterigmata about 20 to 30μ in length.
      3. *A. phoenicis* (Corda) Thom and Church conidia 3 to 4μ, primary sterigmata about 40 to 60μ in length.
      4. *A. pulverulentus* (McAlpine) Thom, conidia 3 to 4μ, primary sterigmata up to 120μ in length.
      5. *A. atropurpureus* A. Zimmermann, conidia 6 to 8.5μ, primary sterigmata up to 40μ in length.
      6. *A. pulchellus* (Speg.) Thom and Church, conidia 8 to 10μ, primary sterigmata up to 30μ in length.
      7. *A. carbonarius* (Bainier) Thom, conidia 8 to 10μ, primary sterigmata up to 120μ in length.

IV. Conidial heads yellow-brown rather than purple-brown or black.
  A. fumaricus Wehmer, conidia 5 to 8μ, primary sterigmata up to 15 by 4μ.

A. niger group: species regarded as synonyms, doubtfully identifiable or inadequately described

S. antacustica Cramer.
A. awamori Nakazawa.
A. batatae Saito.
A. byssoides Sprengel.
S. carbonaria Bainier.
S. castanea Patterson.
S. dipus F. & W.
A. echinosporus Sorokine (not Asp.?).

A. ficuum Hennings.
S. fuliginosa Bainier.
A. fuliginosus Peck.
S. fusca Bainier.
A. fuscus Schiemann.
Aspergillopsis intermedia Speg.
A. japonicus Saito.
S. longobasidia Bainier (?).
Aspergillopsis nigra (Van Tieghem) Speg.
S. nigra Bainier.
Ascophora nigrans.
A. nigricans Wreden.
A. nigriceps B. and C.
A. perniciosus Inui.
A. phaeocephalus Montagne.
S. pseudo-carbonaria Bainier (?).
S. pseudonigra Cost. and Lucet.
Aspergillopsis pulchella Speg.
A. strychni Lindau.
A. subfuscus Johan-Olsen.
A. ustilago Beck.
A. welwitschiae (Bres.) Hennings.

The subheads in this complicated group follow the arrangement used in the synoptical Key.

Conidia with color bars and tubercles breaking up in fluid mounts, hence appearing thin-walled and smooth as commonly examined

A. luteo-niger (Lutz); syn. *S. luteo-nigra* Lutz. (Lutz, L. Trois champignons nouveaux de l'Afrique occidentale. In Bul. Soc. Bot. France 53, p. L. 1906. Collected by A. Chevalier at San Thome, Africa, on fermenting seeds of *Theobroma cacao*; differs from *A. niger* in mycelium golden yellow, conidia smooth.

Latin diagnosis translated: Sterile hyphae lutescent, more or less felted, fertile white, continuous; vesicles spherical, 100μ diameter, fuscous; basidia clavate, radiate 10 to 30μ, frequently bearing 4 sterigmata; sterigmata 4 to 6μ; conidia globose 5μ, in chains, smooth, at first hyaline then fuscous.

Cultures: Lutz; our nos. 4714 and 4729 present conidia smooth when examined in fluid mounts with diffused yellowish-fuscous colors complying with the description of Lutz, but when examined dry, the same conidia are marked with tubercles and bars of coloring substance which may be seen to break in pieces and disappear when water or alcohol is added. Lutz evidently uses the term *basidia* for *primary sterigmata*.

S. fuliginosa Bainier. Bul. Soc. Bot. France 28: 79. 1881. Syn. some variety of *A. niger*. Colonies chocolate in color; sterigmata, primary and secondary equal in length 8.4μ; conidia 4.2μ diameter.

Culture: Probably none; to be dropped.

S. pseudo-carbonaria, nomen nudum found on culture no. 4640.482 from the Bainier Collection; apparently *A. luteo-niger*. Examination shows: Colonies intensely black; heads carbonaceous, crushing readily under the cover-glass; sterigmata in two series both deeply yellowed;

conidia rough tubercular when dry with outer wall and bars of color breaking off in fluid mounts leaving thin-walled smooth cells.

Culture: From the Bainier Collection. Compare *A. luteo-niger*.

A. subfuscus Johan-Olsen. Meddelelser fra Naturh. forening i Kristiania 1885. Cited also in O. Johan-Olsen, Videnskapsselskapets i Kristiania, p. 21. 1886. An organism separated from an *A. fumigatus* culture, by its resemblances to *A. niger*.

As described: Colonies growing best at 37°C., olive-brown in color, but always with a greenish shade; without sclerotia or perithecia; mycelium white with more or less aerial felt; stalks uniform in diameter (? CT.) base to apex, up to 1000 by 15 to 20μ smooth, vesicles 30 to 40μ; sterigmata radiate, up to 20μ long, in single series in cultures, but some secondary sterigmata seen in the original material; conidia globose 3 to 3.5μ diameter, smooth, greenish brown, color extracted by water greenish yellow.

Culture: Johan-Olsen (Sopp), who found it pathogenic to rabbits when injected intravenously.

This form does not appear to have been definitely identified by any other worker; its general appearance as described would call for placing it in or adjacent to the *A. niger* group, excluded only by its smooth conidia, hence possibly related to the forms we have classed with *A. luteo-niger*. The uncertainties involved in grouping have led to the insertion of references leading to the species in, at least, two places in the Key offered.

THE A. NIGER GROUP (MORE STRICTLY)

Stalks smooth; heads in varying shades of fuscous, purplish brown to carbon black; conidia rough or tuberculate from bars or tubercles of brown or purplish brown coloring substance

A. niger Van Tieg. (Van Tieghem, P. E. L. Recherches pour servir à l'histoire physiologique des Mucédinées. Fermentation Gallique. Ann. Sci. Nat. Bot., s. 5, t. 8, p. 240. 1867). =*S. nigra* Van Tieg. (Van Tieghem, P. E. L. Sur le developpement de quelques Ascomycetes. Bul. Soc. Bot. France 24: 102–103. 1877). See also Thom and Currie, Jour. Agr. Res. 7: 1–15. 1916. Described by Van Tieghem as the active agent in fermenting tannin solutions. The name *A. niger* as a designation for a single black Aspergillus is antedated by *Sterigmatocystis antacustica* Cramer for a black Aspergillus from the ear, but has been so widely accepted and especially has been used in so many

biochemical studies upon members of the whole black group that such use is continued here. It would be impossible to determine to-day exactly what organism among hundreds was studied by either Van Tieghem or Cramer. Van Tieghem described his form from material clearly the same as that used by Raulin[1] under the name *Ascophora nigrans*. From a search of the literature antedating Cramer (1859), *A. phaeocephalus* Dur. and Mont., is easily recognizable as one of the series, hence on a strictly priority basis would displace both names. It is not conceivable that the black Aspergilli which are so abundant and conspicuous everywhere escaped the notice of the students of fungi who preceded Montagne, but search of their descriptions and figures fails to show that such organisms were really recognized as Aspergilli unless the tradition that *Monilia pulla* of Persoon's Synopsis fungorum (p. 692) is to be credited as carrying such recognition back to Micheli. Wilhelm[2] discussing *A. niger* expresses the belief that recognition of its identity goes clear back to Micheli's (Nova Plantarum Genera, p. 212. 1729) *Aspergillus capitulo pulla*. This name however practically disappears with Persoon. Definite study of the group as Aspergillus, however, follows the publication of Van Tieghem's paper in 1867. Disregarding priority claims, general usage is deemed adequate reason for continuing the name *A. niger*, but the range of morphology found and of biochemical activities reported justifies warning all users that *A. niger* as ordinarily used does not designate a definite strain or species which can be readily obtained for the purpose of repeating or checking a piece of work, but merely means that the culture was an Aspergillus and that it was "black."

A. niger is, therefore, used as designating a whole group of black Aspergilli with fundamental characters in common within which *A. niger* Van Tieghem becomes the typical species of a section (see Key) falling approximately within the range of detailed characters established by Van Tieghem for the strain used in his fermentation studies.

Characterization of the *A. niger* group (compare plate II, III): Colonies rapidly growing with abundant submerged mycelium colorless or in some strains with more or less yellow color in the hyphae and in the substratum, with aerial hyphae usually scantily produced, but

[1] Raulin, J. Études chimiques sur la végétation des Mucédinées, particulièrement de l'Ascophora nigrans. Compt. Rend. Acad. Sci. [Paris], t. 57, p. 228-230. 1863.

[2] Wilhelm, K. A. Beitr. z. Kennt. Pilzgattung Aspergillus. Inaug. Diss. Wurzburg. 1877.

abundant in age in certain strains; globose, superficial sclerotia produced regularly in certain strains, occasionally in others, not produced under laboratory conditions in most strains; stalks mostly rising directly from the substratum, uncolored or yellow to brown only near the vesicle, smooth, with walls thick without pits but frequently uneven on the inner surface and splitting lengthwise into strips when broken, unseptate or with occasional thin septa, varying greatly in length and diameter in different sections of the group and in colonies on different media or even in sections of the same colony, thus ranging from strains with stalks 200 to 400μ by 7 to 10μ to forms with stalks several millimeters long and 20μ or more in diameter; conidial heads fuscous, blackish brown, purple brown, in every shade to carbonaceous black apparently varying in intensity with the quantity of coloring matter produced, varying from small almost columnar masses of a few conidial chains to the more common globose or radiate heads, commonly up to 300, 500μ or occasionally 1000μ in diameter with periphery variously splitting into radiating conidial columns; vesicles globose or subglobose, thick-walled commonly 20 to 50μ, occasionally up to 100μ in diameter, colorless or more commonly more or less intensely yellow-brown; sterigmata in one series in a few strains or species, and in young colonies and small heads in many species, but typically in two series in mature average sized heads, colorless at times, usually more or less intensely brown, even carbonaceous; primary sterigmata closely covering the vesicle, varying greatly in size in the same colony but usually within limits for each strain, from a minimum of about 10 to 15μ to a maximum of about 120μ in length in *A. carbonarius*; secondary sterigmata more uniform, ranging usually 6 to 10μ by 2 to 3μ, usually more or less brown to almost black; conidia when ripe globose, with walls thin in some forms, to fairly thick, at first smooth with diffused brown or fuscous color then rough or spinulose from coloring substance deposited as tubercles or bars or loops between the outer or primary wall and the inner or secondary wall, mostly 2.5 to 4μ, less frequently 5μ in diameter in the commoner strains, in occasional forms doubled or trebled to 6, 8 or even 10μ in diameter.

The differences in measurements reported have been more or less correlated by Haenicke[3] with cytological differences, which when fully understood may lead to the establishment of certain stable species. In examining exsiccati from several herbaria and in many hundreds of cultures from many parts of the world, a very complete series of variations in color and measurement is the basis of the group characterization

[3] Haenicke, A. Zeitschr. Bot. 8: 225-352. 1916.

proposed. The range of biochemical activities indicated in numerous papers dealing with members of the group appears to be equally wide. We appear to be dealing with a cosmopolitan, almost omnivorous group of races or species which show great variability with probably many natural or induced mutations. This was indicated by Schiemann[4] in describing *A. cinnamomeus* and *A. fuscus*, and is amply justified by the range of morphology found in races which, while having most of their characters in common, maintain minor differences over long periods of time in comparative culture.

Sclerotium production regularly occurs in some sections of the great group, occasionally in strains or species throughout the whole group. Brefeld in Botanische Zeitung 34: 265, 1876, reports that these sclerotia produce ascospores after a long period, but gave no further information. Dangeard (loc. cit., see p. 21) reports practically the same observations, but these reports have not been verified by others.

Aspergillopsis nigra (Van Tieg.) Speg. Myc. Arg. V, in An. Mus. Nac. Buenos Aires, ser. 3, t. 13; p. 435. 1911. Syn. *A. niger* Van Tieghem. The authors do not regard the presence of the pigment in the spores of the black Aspergilli as adequate basis for a separate genus.

Conidia with color bars or tubercles persistent
  Sterigmata in 1 series
    Conidia globose
      1. Conidia 3μ, sterigmata 15μ in length.....*A. nanus* Mont.
      2. Conidia finely roughened 4 to 5μ
        Without sclerotia.....*A. luchuensis* Inui.
        Without sclerotia.....*A. perniciosus* Inui.
        With sclerotia.....*A. japonicus* Saito.
  Sterigmata in 2 series
    Conidia globose or subglobose, the remainder of....The *A. niger* group.
    Conidia ovoid 3.3 to 5 by 5 to 6.5μ.....*A. violaceo-fuscus* Gasp.

A. nanus Montagne (Montagne, J. F. C. Sylloge Generum Specierumque Cryptogamarum . . . . p. 300, no. 1112, Paris. 1856. Sacc. Syll. 4: 71. 1886).

The description clearly distinguishes this organism as a member of the black group and as having a single series of sterigmata about 15μ in length and spores 3μ in diameter. While such a species is not impossible, heads with this morphology are very common in some strains of the black Aspergilli, especially in young colonies which have just

[4] Schiemann, E. Ztschr. Induktive Abstam. u. Vererbungslehre 8: 1–35. 1912.

begun to produce conidia. The same colonies show the double series of sterigmata when further developed. We are inclined to question whether a form with the single series of sterigmata alone is to be found.

Culture: On cooked rice, Montagne; *A. niger* group.

A. luchuensis Inui, T. Untersuchungen über die niederen Organismen welche sich bei der Zubereitung des alkoholischen Getränkes "Awamori" betheiligen. Jour. Col. Sci. Imp. Univ. Tokyo 15: 469, pl. 22, fig. 1–8. 1901. See also Usami, K. Mykologische Notizen über Awamori-Koji-Pilze (Aspergillus) und Rhizopus Delemar. Centralb. Bakt. etc., 2 Abt., 43, p. 250. 1915. Compare plate III, 4666.

This form differs from *A. niger* in showing a single series of sterigmata 7 to 9 by 5μ, but shows the colors of *A. niger*, similar stalk and vesicle, with conidia 4 to 4.5μ and finely roughened. Inui gives: Stalks 1 to 2 or 2.5 mm. by 10 to 15μ, wall heavy, smooth, hyaline or brownish; vesicles 20 to 30μ to 40μ (CT) diameter, showing pores or markings when sterigmata fall off; sterigmata in one series 3 by 6μ; some branched sterigmata were found by us in Hanzawa's culture; conidia 4 to 4.5μ in diameter, finely roughened (3.5μ mostly and with spiny points not bars of color CT.).

Culture and biochemical study: Inui; Usami; Thom no. 4202.18c from China and no. 4291.3 from Hanzawa; N. O. 3 from Louisiana.

A. perniciosus Inui. Jour. Col. Sci. Imp. Univ. Tokyo 15: 473. 1901. Inui recorded a yellowish greenish color in the mycelium of this species which is regarded as related to *A. luchuensis* and *A. niger*. The description given is probably inadequate to separate the species unless it can be again collected from Awamori Koji in direct relationship to *A. luchuensis* and restudied.

As described: Mycelium with a yellow-greenish (?) color; heads white to yellow to yellow brown; stalks unseptate, up to 2500μ long, smooth; sterigmata in one series, shorter than those in *A. luchuensis* (which are 6μ long); conidia globose, rough, 4 to 5μ diameter.

Culture: Not completely reported; not seen by us.

This form may be arbitrarily placed next to *A. luchuensis* as suggested by Inui, since it is very doubtful if true green color is indicated by Inui's description.

A. japonicus Saito (Saito, Kendo. Nachtrag zu der Abhandlung "Untersuchungen über die atmosphaerischen Pilzkeime, I." Bot. Mag. [Tokyo] vol. 20, no. 233, p. 61, 5 fig. 1906).

Saito's organism with the colors of *A. niger* and *A. luchuensis* has the stalk wall described as coarsely or finely roughened but is figured as

showing scattered concretions without pits, and with simple sterigmata and the development of sclerotia 650 to 1000μ in diameter.

As described: Colonies white to black-brown; stalks 130 to 610 by 13μ with wall 2 to 3μ thick, granular but not pitted; vesicles 20 to 45μ, fertile all over; sterigmata 7 to 9 by 5μ; conidia 4 to 5μ globose, figured as spinulose, slowly black-brown.

Culture: Saito. No culture with all of these characters has been found by us, but the figures given justify belief that the species belongs with the sclerotium forming group in this series. In this group we have two cultures, nos. 4030.3 and 4030.5, with approximately the measurements of *A. japonicus*. Granules or concretions on the stalk wall are occasionally found in *A. niger* and may account for the roughenings described by Saito, since this type of roughening corresponds well with the figures given.

A. violaceo-fuscus Gasper. (Gasperini, G. Sopra un nuovo morbo che attacca i limoni e sopra alcuni ifomiceti. Atti Soc. Toscana Sci. Nat. Pisa, Mem. 8, fasc. 2, p. 326. 1887).

By description this is a variant member of the great group of black Aspergilli with short sterigmata and elliptical conidia; stalks unseptate, hyaline, about 2 mm. by 12 to 18μ; heads about 95μ in diameter, globose; vesicles 42 to 57μ globose; sterigmata radiate, primary 6 to 8μ long, secondary 2 to 4μ long; conidia ovoid 3.26 to 5 by 5 to 6.5μ, at first hyaline then violaceo-fuscous, verruculose, perithecia or sclerotia not found.

Culture: Gasperini; grows readily on many substrata.

Three cultures, no. 3522.30 from Porto Rico, no. 4725.688 from Jamaica, and no. 4724.44 from Raistrick in England, approach the description given by Gasperini, varying mainly in somewhat smaller conidia. Without having seen Gasperini's material, we think it probable that these may be correctly grouped as strains of *A. violaceo-fuscus*.

Conidia 2.5 to 4μ; primary sterigmata usually 20 to 30μ long, in mature average sized heads. Colonies fuscous; conidia smooth or very delicately marked..... *A. schiemanni* Thom

Colonies brown, purple brown to black; conidia globose, rough (see group characterization)..... *A. niger* sense of Van Tieghem

A. schiemanni (Schiemann) Thom. Jour. Agr. Res. 7: 13. 1916. Syn. *A. fuscus* Schiemann (Schiemann, Elisabeth. Mutationen bei Aspergillus niger Van Tieghem. Ztschr. Induktive Abstam. u. Vererbungslehre, Bd. 8, Heft 1/2, p. 1–35, 16 fig., 2 pl. (1 col.). 1912). A new combination was necessary because the specific name fuscus had

been already used by Bonorden for a species of Aspergillus and fusca by Bainier for a species of Sterigmatocystis. This form is distinguished only by its fuscous conidia which are smooth or very delicately roughened.

Culture: By Schiemann. Our 3534c received from Schiemann and maintained in culture without loss of characters for at least twelve years.

A. fuscus Schiem. (Schiemann, Elisabeth. Mutationen bei Aspergillus niger Van Tieghem. Ztschr. Induktive Abstam. u. Vererbungslehre, Bd. 8, Heft 1/2, p. 1–35, 16 fig., 2 pl. (1 col.). 1912.) Syn. *A. schiemanni* Thom. This mutant from *A. niger* is distinguished by the color and smoothness of its spores from the parent strain. The name is invalidated by the occurrence of the names *A. fuscus* Bonorden and *S. fusca* Bainier.

Culture: Schiemann, No. 3534c received from Schiemann.

A. awamori Nakazawa. Nomen nudum. A culture so labeled was received by us from Hanzawa (4291.2) and belongs with *A. schiemanni* (*A. fuscus* Schiemann).

Culture: Hanzawa, 4291.2.

Species described as black Aspergilli without adequate information to separate them from A. niger Van Tieghem. Unless further work should render such forms as A. batatae commercially important with adequate comparative studies to render identification certain, these names can hardly be maintained.

A. batatae Saito (Saito, Kendo. Mikrobiologische Studien über die Zubereitung des Batatenbranntweines auf der Insel Hachijo (Japan). In Centralbl. f. Bakt. etc. 2 Abt., 18, No. 1/3, p. 34. 1907).

Saito's organism as described is close to *A. niger*; with colonies reaching brownish-black through yellow shades; stalks 2 to 4 mm. by 12 to 20μ, smooth, thick-walled, brown in upper portion; vesicles 35 to 50μ globose, radiate; sterigmata primary 24 to 40 by 8μ at apex, secondary 10 by 3.2μ; conidia globose, brown, finely roughened, 4 to 5μ diameter. No sclerotia found but enlarged sterile cells resembling those about the perithecia of *A. nidulans* are reported.

Culture and biochemical study: Saito; not seen by us.

A. byssoides. In Sprengel Sys. Veg. ed. 16, 4, p. 541. 1827. A fungus on rotting paper with globose fuscous heads. This might have been *A. niger* or one of several other organisms. It should be dropped.

Culture: None.

S. castanea Patterson. (Patterson, Flora W. New species of fungi. Bul. Torrey Bot. Club 27: 284. 1900.) The type appears to have been lost, but examination of material determined by Mrs. Patterson under this name in 1901 in the Pathological Collections of the Bureau of Plant Industry at Washington shows a strain of the *A. niger* group with conidia mostly 3.5 to 4μ but occasionally 4.5μ in diameter.

Culture: None.

A. ficuum (Reich.) Henn. (Hennings, P. *Ustilago Ficuum* Reich. = *S. Ficuum* (Reich.) P. Henn. In Hedwigia, 34, 2, p. 86. 1895.) = *Ustilago ficuum* Reich. (Reichardt, H. W. Ein neuer Brandpilz. In Verhandl. K. K. Zool. Bot. Gesell. Wien, 17: 335. 1867). Regarded as *A. niger* by Wehmer (Wehmer, Carl. Zur Kenntnis einiger Aspergillus-Arten. In Centralbl. f. Bakt. etc. 2 Abt., 18, No. 13/15, p. 394–395. 1907). See also Thom and Currie, Jour. Agr. Res. 7: 1–15. 1916. Slight differences in morphology are reported by Hennings, but disregarded by Wehmer.

Culture: No. 142 received from Westerdijk under this name is reported by Thom and Currie as the most rapid producer of oxalic acid of all strains of *A. niger* tested.

Amons (Amons, W. J. Th. Archief Suikerind. Nederlandsch-Indië, Jaarg. 29, Deel 1: 1–7. 1921) uses this name for an Aspergillus black with a violet tinge found in deteriorating sugar giving the following: Reverse of colonies colorless or light yellow; conidiophores 1 to 1.5 mm. by 10 to 12μ, colorless, smooth; vesicles 42 by 50μ or globose; sterigmata, primary about 9μ, secondary about 6μ in long axis; conidia oval averaging 3.6 by 4.5μ at first then globose 4μ and finely roughened. Amons does not report sufficient study of his organism to insure its identification.

A. fuliginosus Peck (Peck, C. H. Descriptions of new species of fungi. In Bul. Buffalo Soc. Nat. Sci. (1873/74) 1: 69. 1874; also, Peck, C. H. Report of the botanist. In 26th Ann. Rpt. N. Y. State Mus. Nat. Hist. (1872), p. 79. 1874). No valid data are offered for separating this strain from *A. niger*. Specimens under this name have been examined as follows: From Newfield, N. J., and No. 2505 herb. A. Commons with conidia 4 to 4.5μ, Bartholemews No. 1566 and probably the same material in E. and E. Fungi Columbiani No. 1492, both in the Ellis Collection at the N. Y. Bot. Garden; T. A. Williams Nebraska Flora No. 392 in Path. Coll. U. S. Dept. Agr.; all are *A. niger*.

Culture: None.

A. nigricans Wreden (Wreden, Robert. Recherches sur deux nouvel-

les espèces de végétaux parasites (Aspergillus flavescens et Aspergillus nigricans) de l'homme. In Compt. Rend. Acad. Sci. [Paris] 65: 368. 1867). This is regarded as *A. niger* by Wilhelm (Wilhelm, K. A. Beiträge zur Kenntniss der Pilzgattung Aspergillus, p. 70. Strassburg-Berlin, 1877. Inaug. Diss.), by Siebenmann (Siebenmann, Friedrich. Die Fadenpilze Aspergillus flavus, niger u. fumigatus; Eurotium repens (u. Aspergillus glaucus) und ihre Beziehungen zu Otomycosis Aspergillina, p. 82. Wiesbaden, 1883. Inaug. Diss.); and by Wehmer (Wehmer, Carl. Zur Kenntnis einiger Aspergillus-Arten. Centralbl. f. Bakt. etc. 2 Abt., 18, No. 13/15, p. 394–395. 1907). While probably correctly placed in the *A. niger* group, the absence of adequate description or identifiable material justifies the rejection of this and the acceptance of Van Tieghem's specific name as clearly established.

Culture: Inadequate or none; to be dropped.

A. nigriceps B. and C., in the Curtis Collection. Specimen collected by Charles Wright (no. 927) in Cuba. Cited by Cooke, M. C. Grevillea 17: 21, 1888. A slide from this material prepared by Bullard and preserved in the Harvard Collection shows a characteristic organism of the *Aspergillus niger* series, not separable from *A. niger* Van Tieghem.

Culture: None; to be dropped.

S. pseudonigra Costantin and Lucet (Costantin and Lucet. Sur le Sterigmatocystis pseudonigra. In Bul. Soc. Mycol. France 19: 33–44. 1903). This is regarded as A. niger by Wehmer (Wehmer, Carl. Zur Kenntnis einiger Aspergillus-Arten. Centralbl. f. Bakt. etc. 2 Abt., 18, No. 13/15, pp. 394–395. 1907). The distinctions proposed by the authors are based upon pathogenicity and cultural reactions, not upon definite morphological characters.

Conidia 3 to 4μ; primary sterigmata about 50μ in length (40 to 60μ,): type of series..... *A. phoenicis* (Corda) Thom.

Cultures and exsiccati with these measurements fairly regularly produced are frequent enough to justify belief in the existence of a series of strains intermediate in measurements of sterigmata between the typical *A. niger* Van Tieghem and gigantic forms such as *A. carbonarius* Bainier.

A. phoenicis (Corda) Thom. See Thom, C., and Currie, J. N. Aspergillus niger group, Jour. Agr. Res. 7: 11. 1916.

In describing the black Aspergillus as found upon dates, Patouillard and Delacroix compared this material to specimens in the museum labeled "*Ustilago phoenicis*" and attributed to Corda, thus establishing the identity of the organism of Corda, which was not recognizable from

any previous references. *A. ustilago* Beck is described with the same measurements. The measurements of sterigmata place *A. phoenicis* in the section of the group with primary sterigmata 50 to 60μ in length; it was described as *Ustilago phoenicis* Corda, Icones Fung. IV., p. 9, pl. 3, fig. 26. 1840, and transferred by Patouillard and Delacroix as noted above to *S. phoenicis* (Corda) Patouill. and Delacr. (Patouillard, Narcisse, and Delacroix, Georges. Sur une maladie des dattes produite par le Sterigmatocystis phoenicis (Corda) Patouill. et Delacr. In Bul. Soc. Mycol. France 7: 119, pl. 9. 1891.) If we accept the use of "phoenicis" attributed to Corda as correct this species becomes the type of the section of the black Aspergilli with intermediate length of primary sterigmata and conidia not over 4μ in diameter. This was cited by Thom and Currie as *A. phoenicis* (Corda) Pat. and Delacr.

S. antacustica Cramer (Cramer, Carl. Ueber eine neue Fadenpilzgattung: Sterigmatocystis, Cramer. Vrtljschr. Naturf. Gesell. Zürich, Jahrg. 4, Heft 4, p. 325 taf. II, figs. 1–15. 1859). Described and figured from material from the external ear of man. The original material was carefully described but not cultured, hence while clearly attributable to the *A. niger* group, it can not be certainly placed in a particular section of the group from measurements based upon the pathological specimens used.

As described: Vesicles 45μ in average diameter; sterigmata, primary 20 to 50μ long, secondary not measured but reported as several in each group; conidia 2.5 to 3.5μ. Culture no. 685 in Rabenhorst's Fungi Europaei Ed. Nova, ser. II, cent. VII, marked "in solution acidi tannici, leg. C. Cramer"—has stalks up to 15μ in diameter with walls 2μ, heads up to 380μ in diameter, primary sterigmata 107 by 8.5μ, brownish, more or less septate; conidia 2 to 4.5μ, marked as in *A. niger*. If the specimen in the Rabenhorst series is correctly attributable to Cramer, the name *S. antacustica* might apply to the black Aspergillus with very long primary sterigmata and conidia 3 to 4μ in diameter. These forms are much less common than the morphology given by Van Tieghem for *A. niger*, which has been accepted too widely to warrant displacement in designating that section of the group.

Cramer left no record of cultivation of *S. antacustica* from the original material, hence even if the specimen grown in tannin solution was handled by him, the measurements are an uncertain basis for grouping the original material, which as examined had primary sterigmata up to 50μ long, hence would automatically belong with *A. phoenicis* as named by Corda.

A. phaeocephalus Durieu and Montagne, Fl. Alg., p. 342. Syn. *S. phaeocephala* (Dur. and Mont.) Saccardo, Fungi italici fig. 903, described from onion bulbs, and no. 1244 in Sacc. Myc. Veneta, from cheese and moist paper, 1877.

Saccardo interprets this form as one of the black Aspergilli with the double series of sterigmata, from the data given by Montagne: Stalks up to 2 mm. long by 13μ in diameter, colorless below, somewhat reddish brown above, constricted below the globose vesicle; vesicles up to 50μ in diameter; sterigmata, designated "basidiomorphis," hence probably in 2 series, up to 60μ long, radiating uniformly; conidia colorless then yellowish to fuscous (black to the naked eye).

Exsiccati: Saccardo no. 1244 Myc. Ven. is one of the *A. niger* group as examined by us.

Culture: None.

If a specific name is to be given to the black Aspergilli with primary sterigmata 50μ long, it may be a question whether this name should be used or *A. phoenicis*.

Aspergillopsis intermedia Speg., Myc. Arg. V. An. Mus. Nac. Buenos Aires, ser. 3, t. 13; p. 435. 1911. A member of the *A. niger* group with primary sterigmata about 40 to 50μ in length and conidia 4 to 4.5μ in diameter. Apparently never studied in culture. Such measurements as we have from Spegazzini's description upon a special natural substratum are not adequate ground for separating such a species, but from the information given it is tentatively placed in the *A. niger* group near *A. phoenicis*.

A. longobasidia Bainier, nomen nudum. Culture so labeled in Bainier Collection, our no. 4640.477. When examined, this culture showed very large heads, intensely black, with primary sterigmata 40μ long, and conidia very black and coarsely tuberculate about 4.5μ (central body 3.5μ becoming 4.5μ from the coarse tubercles).

Culture: Bainier Collection no. 4640.477 is a member of the section of *A. niger* with *A. phoenicis* and *A. phaeocephalus*.

S. nigra Bainier. Syn. for *A. phoenicis* (Corda) Thom, in the *A. niger* series. Described from a gall nut, citing Van Tieghem's description of *A. niger*, and giving primary sterigmata 46μ long, and conidia echinulate black-brown, 4.2μ in diameter. This clearly belongs to the section of the *A. niger* series with primary sterigmata about double the length of the type species.

Culture: Not reported.

A. ustilago Beck (Wawra, Heinrich. Itinera Principum S. Coburgi,

t. 2, p. 148. Wien, 1888. Sacc. Syll. 10: 526, 1892). The description relates this form to the section of the *A. niger* group showing sterigmata 50μ or more in length: fuligineus; stalks 500 to 800μ, striatulate, thick-walled; vesicle 75μ; basidia (?) 50μ long; spores mostly globose or subglobose, verruculose, 2.5 to 4.9μ; on pericarp of *Phyllanthus emblica* L., East Indies.

S. welwitschiae (Bresadola) Hennings. (Wehmer, Carl. Zur Kenntnis einiger Aspergillus-Arten. In Centralbl. f. Bakt., etc., 2 Abt., 18, no. 13/15, p. 394–395. 1907.) Syn. *Ustilago welwitschiae* Bresadola.

No data are reported which warrant the establishment of a separate species. Examination of material from Hennings by Wehmer (Centralbl. f. Bakt., etc., 2 Abt., 18, no. 13/15, p. 394–395. 1907) furnished no basis for separating the form from *A. niger*. A similar specimen in the Harvard Herbarium shows primary sterigmata up to 51 by up to 12μ, which would place it in the section with *A. phoenicis*.

Culture: Not reported.

Conidia 3 to 4μ

Primary sterigmata up to 120μ in length, type *A. pulverulentus* (McAlpine) Thom

Synonym..... *A. strychni* Lindau

A. strychni Lindau (Lindau, Gustave. Aspergillus (Sterigmatocystis) strychni nov. spec. Hedwigia, Bd. 43, Heft 5, p. 306–307. 1904). Syn. for *A. pulverulentus* Thom. This is regarded as *A. niger* by Wehmer (Wehmer, Carl. Zur Kenntnis einiger Aspergillus-Arten. In Centralbl. Bakt., etc., 2 Abt., 18, No. 13/15, p. 394–395. 1907).

Lindau's material seems to be represented by no. 193 in Kabát et Bukák, Fungi imperfecti exsiccati, a specimen of which is in the Pathological Collections of the U. S. Dept. of Agr. Examination of this material shows an organism with conidia 4μ in diameter marked as in *A. niger*, with stalks and heads much as in *A. niger*, except that the primary sterigmata reach a length of 120μ as described by Lindau for this species, for *S. pulverulenta* by McAlpine, and as found by us in the Rabenhorst specimens of *S. antacustica* and specimens labeled *A. welwitschiae*.

The details of structure found in this form appear in a culture (no. 2580) obtained in a study of red peppers (Capsicum species) from Barcelona, Spain. The interior of these fruits had been destroyed and there remained a shell containing a mass of Aspergillus spores. While, perhaps, not common, forms with this morphology will be occasionally encountered.

Exsiccati: Basis of Lindau's description.

A. pulverulentus (McAlpine) Thom. See Thom and Currie, Jour. Agr. Res. 7: 10–11. 1916; syn. *S. pulverulenta* McAlpine. (McAlpine, Daniel. Australian fungi. Agr. Gaz. N. S. Wales (1896) 7: 302. 1897).

McAlpine gives: "White to dirty yellow mycelium, producing numerous, slender, outstanding colourless threads, surmounted by small, black, rounded heads (155 to 340μ diameter) and covering nearly entire surface of pod with a black soot-like powdery mass. Mycelium creeping composed of slender, septate, branched, hyaline hyphae, 3 to 6μ broad. Fertile threads erect, stiff, standing out like so many grey hairs, non-septate, unbranched, clear, but yellowish-brown and tapering towards top, variable in length, up to 7 mm. long and 20μ broad, thickish-walled (5μ), much narrower and thinner-walled towards base. Top of fertile branch inflated, forming globular, sub-globular or oval vesicle 80 to 170μ diameter, studded all over with close-set round markings, whence basidia proceed. Radiating, yellowish-brown basidia from vesicle, usually septate at least once, swollen at outer end, up to 144μ long and usually 8μ broad, bearing sterigmata. Sterigmata numerous (10 or more) pale yellow, stout, oblong to awl-shaped, projecting peg at outer end separated by a septum and bearing chains of gonidia at least 4 deep, average 14 to 18μ long. Conidia spherical, deep brown or pallid, decidedly warty, average 4μ diameter." This form belongs in the section of the *A. niger* group with very long primary sterigmata without change in diameter of the conidia.

Conidia larger than 3 to 4μ in diameter
Primary sterigmata not over 40μ in long axis; conidia 6 to 8.5μ; type of series.....*A. atropurpureus* A. Zimmermann
Synonym.....*S. fusca* Bainier
Synonym.....*S. dipus* F. & W.
Conidia 5 to 8μ; yellow brown colonies
A. fumaricus Wehmer ? Neuberg's culture 4668.2
Conidia 8 to 10μ.....*Aspergillopsis pulchella* Speg.
Primary sterigmata up to 120μ long; conidia 8 to 10μ
A. carbonarius (Bainier) Thom.

A. atropurpureus A. Zimm. (Zimmermann, A. Ueber einige an tropischen Kulturpflanzen beobachtete Pilze. II. In Centralbl. f. Bakt., etc., 2 Abt., 8, no. 5/7, p. 218. 1902.)

Stalks hyaline or somewhat brownish in age, up to 800 by 16 to 20μ; heads (vesicles ?CT.) 60 to 80μ hyaline to brown; sterigmata, primary 16 by 6μ, secondary 3 to 4 by 1.5 to 2μ; conidia globose rough with

prominent warts, purplish black 6 to 10μ diameter. On *Coffea liberica*, in Java.

Culture: None. Type material of this species has not been seen, but as described it is close to *S. fusca* Bainier.

Culture 4724.44 contributed by Raistrick under this name differs in conidial size from the description of Zimmermann. This culture had been obtained from Baarn and by that laboratory from Blochwitz. In biochemical reactions, 4724.44 is said by Raistrick to be allied with the *A. niger* group; in morphology it belongs in the section with *A. luchuensis*.

The three names, *S. fusca* Bainier, *A. atropurpureus* A. Zimmermann and *S. dipus* F. & W., are attached to black Aspergilli, with double sterigmata and conidia about twice the range of diameter found in *A. niger* Van Tieghem. We have seen Bainier's material and have living cultures with this range of diameter of conidia. Since *S. fusca* Bainier is untenable on account of Bonorden's species, *A. atropurpureus* becomes available as designating this section of the group of black forms.

S. fusca Bainier, Bul. Soc. Bot. France 27: 29, pl. I, fig. 5. 1880; compare Roumeguère Fungi gallici exsiccati no. 995 grown upon most bread in Toulouse 1880; compare Sartory, A., et Jourde, A. Compt. Rend. Soc. Biol. [Paris] 64: 926–928. 1908.

Bainier's description and Roumeguère's exsiccati are dated 1880, and agree in presenting an organism belonging to the *A. niger* group with measurements approximating the common black Aspergillus, except in having conidia about double the size described for *A. niger* and slightly smaller than those of *A. carbonarius*, according to Bainier rarely exceeding 9.4μ in diameter, in the exsiccati as examined by us 5 to 8.5μ, marked as in *A. niger* and *A. carbonarius*. A culture with approximately the same spore measurements has been sent to us by Fonseca from Brazil (4707.878). There appears, therefore, to be a black Aspergillus corresponding with Bainier's description; Sartory and Jourde, 1908, however, clearly describe a culture under this name which belongs with *A. tamarii*. The close association of Sartory with Bainier in 1908, furnished possibly special opportunities for verification of the usage of the name *S. fusca*, but we are inclined to place the species along with its apparent synonym *S. dipus* in the *A. niger* group, leaving *S. fusca* in the sense of Sartory and Jourde as a synonym of *A. tamarii*.

S. dipus Ferdinandsen and Winge, Bot. Tids. 30: 220, fig. 6. 1910. Found on spoiling *Theobroma cacao* in Venezuela. The stalks, heads

and conidia described ally this with *S. fusca* Bainier. The footcell described as basis of the name is characteristic of the genus, not specific.

Culture: Not reported. This organism is not separable from other forms in this section of the *A. niger* group.

A. fumaricus Wehmer, in Ber. Deut. Chem. Gesell. 51, no. 14, pp. 1663–1668, figs. 1–6. 1918. This species has been named but not fully described and not distributed by Wehmer. Its biochemical activity as a producer of fumaric acid is covered in the paper cited together with the admission that it belongs to the *A. niger* group. Culture no. 4668.2 received from Neuberg under the name presents a variant strain in that group which may be described: Colonies producing a mass of yellow mycelium; stalks scantily and tardily produced up to 1, 2 or 3 mm. long, by 20 to 22μ in diameter, smooth, bearing large radiate, yellow-brown heads; vesicles up to 60 by 100μ in diameter, with walls thin, easily crushed; primary sterigmata up to 15 by 4 to 5μ; secondary sterigmata rather coarse, some growing out into aborted hyphae; conidia commonly 5μ, occasionally 7 to 8μ in diameter, with lengthwise color bars as in *A. niger* but paler in color.

Culture: Wehmer.

Note: No. 4668.2 from Neuberg is tentatively accepted as correctly named.

A. pulchellus (Speg.), *Aspergillopsis pulchella* Speg., Myc. Arg. V, in An. Mus. Nac. Buenos Aires, ser. 3, t. 13; p. 436. 1911.

Colonies intensely black; stalks 1 to 2 mm. by 18 to 20μ, darkened; vesicle 50 to 60μ in diameter, fumose; sterigmata primary 30 by 10μ, colored; conidia 8 to 10μ in diameter, figured as rough. There are discrepancies between Spegazzini's description and his figures, but taken together they indicate a black Aspergillus with ordinary measurements of *A. niger*, except for the large conidia 8 to 10μ diameter.

Culture: None; not seen by us.

A. carbonarius (Bainier) Thom. (In Thom, C., and Currie, J. N. The Aspergillus niger group. Jour. Agr. Res. 7: 12. 1916; syn. *S. carbonaria* Bainier.)

Colonies grown in Czapek's solution agar show vegetative mycelium white or with some yellow in submerged areas, broadly spreading, more or less zonate; sclerotia produced upon the surface of the substratum in old cultures; fruiting areas carbon-black; stalks colorless below, yellow to yellow-brown toward the apex, 4 to 6 mm. or longer and up to 25μ in diameter, with walls smooth, up to 4μ in thickness; heads globose varying in diameter up to 500μ; vesicles up to 90μ in diameter, fertile

over entire surface, commonly with contents yellow-brown to black and in old heads forming with the primary sterigmata a hard, brittle, carbonaceous mass; sterigmata in two series, primary sometimes 1-septate, from 20 to 40μ long in young or small heads and up to 120μ long in large heads by 5 to 13μ in diameter at the apex, secondary 8 to 14μ by 3 to 6μ; conidia at first smooth becoming rough when ripe, 5.5 to 10.5μ in diameter. Colonies grow well upon all culture media used, with temperature optimum below 37°C. Growth at 37°C. slow and more or less dwarfed. A culture from Dr. Blakeslee, no. 4030.1, reproduces the morphology recorded by Bainier.

Culture: Bainier, Thom, no. 4030.1. Collections: Farlow.

Blakeslee's culture reproduces the description given by Bainier in detail. The same morphology was also found in Farlow's specimen. There is, therefore, no question as to the occasional occurrence of this large spored form which probably represents a mutant from the usual type of black species.

S. carbonaria Bainier (Bainier, Georges. Sterigmatocystis et Nematogonum. In Bul. Soc. Bot. France 27 (s. 2, t. 2), p. 27–28. 1880). Syn. *A. carbonarius*.

SECTION XI

The Yellow, Ochraceous, and Brown Aspergilli

Three groups with connecting forms and forms of doubtful relationship are arbitrarily placed here.

Stalks smooth (free from pits); mature heads brown.....*A. wentii*
Stalks pitted
  Heads yellow to ochraceous.....*A. ochraceus* group
  Heads brown when mature.....*A. tamarii* and allies

A. wentii Wehmer. Centralbl. f. Bakt. etc. 2 Abt., 2, p. 150. 1896. See also Wehmer Monogr. Wehmer's culture appears to have been deposited with the "Centraalstelle" then at Amsterdam from which we received it (our number 116). Subsequently, many isolations from widely separated places in America and Asia show that strains belonging in a group with that described by Wehmer are not uncommon.

The following characterization from our own culture work is essentially in harmony with Wehmer's description(see Thom, C., and Church, M. B., Am. Jour. Bot. 8: 116–117. 1921); compare plate II, 116; Colonies on Czapek's solution agar with cane sugar, deeply floccose, spreading, with sterile hyphae white or yellowish, and with heads white at first, changing through cream, cream buff, honey yellow, old gold, to light brownish olive, medal bronze, or in old cultures sometimes snuff brown (Ridgway, column 19, plates IV, XVI, XXX, and plate XXIX, 15″ k; recorded as coffee-brown to chocolate brown by Wehmer), and in some strains producing large masses of aerial mycelium which in tubes may fill the lumen 3 cm. above the substratum; reverse of colony yellowish at first, becoming reddish brown when old; agar frequently colored yellow; stalks 2 to 3 mm. or up to 5 mm. long, commonly 10 to 12μ or sometimes up to 25μ in diameter, inconspicuously 1- to 2-septate, with walls colorless, up to 4μ in thickness, and smooth, often studded with droplets in young cultures, enlarged at tips to vesicles widely varying up to 80μ in diameter; heads large, yellow to brown, radiate (or globose); sterigmata usually in two series, primary varying greatly, 6 to 8μ, occasionally to 15μ by 3 to 5μ, in extreme cases up to 60μ by 8 to 10μ; secondary 6 to 8 by 3μ (a single series is recorded by Wehmer 15 by 4μ). Conidia pyriform to globose, usually about 4 by 5μ, less commonly up to 5 to 5.5μ by 5 to 6μ (4.2 to 5.6μ,

Wehmer), with walls thickened to leave pits or furrows on the surface arranged roughly lengthwise of the spore chain, frequently appearing smooth or nearly so with low magnifications, commonly more or less plasmolyzed when treated with 95 per cent alcohol. No perithecia have been found. Sclerotia have been occasionally but not uniformly produced.

S. aerea Bainier, Bul. Soc. Bot. France 28: 78. 1881. Colonies bronze in color, stalks up to 1 cm. by up to 21μ; vesicle thick-walled showing marks and pits when sterigmata are detached; sterigmata, primary up to 42μ and secondary to 12.6μ long; conidia globose, smooth 4.2μ.

Culture: Not reported.

Since the description is incomplete and the organism was not afterward reported, it has been tentatively placed between *A. wentii* and *A. tamarii* on account of color and general proportions as described.

THE A. OCHRACEUS GROUP

Stalks with walls pitted (compare p. 101), sometimes also granular, often reported as rough or spinulose when observed with low magnifications; pitting only partially or with difficulty detectable in a few forms

1. Colonies never green (compare *A. flavus-oryzae* group, p. 198)
   Colonies when mature in shades of brown, yellow brown, orange, or umber, p. 183 and 193
   Colonies some shade of pale yellow (such as sulphur yellow) to ochraceous (never brown or umber).....The *A. ochraceus* group

Summary of characters of the *A. ochraceus* group:

Stalks with outer layer of walls yellow and pitted, commonly also showing irregularly distributed concretions or warts; pitting of walls indistinct, difficultly detected or suppressed in a few strains which are otherwise obviously related; conidia thin-walled, smooth or delicately echinulate, scarcely roughened with color bars; showing transition toward the next group in *S. delacroixii* and *A. ostianus* (compare p. 163 and 193)

Group characterization: The colony appearance of this group of Aspergilli varies greatly with presence or absence of sclerotia. Some species are typically ochraceous in color from abundant conidial heads and are not known to produce sclerotia; some produce few sclerotia or clusters of sclerotia under very special conditions still showing abundant conidial heads; others always produce sclerotia varying the abundance from few and scattered globose or elliptical bodies up to 0.5 mm. or more in long axis in a colony still characteristically ochraceous in color from abundant conidial heads, to colonies whose whole surface is densely covered, even piled with sclerotia in shades of yellow to orange vinaceous, or in purplish tones, among which few and single,

clustered, or irregular patches of conidia-bearing stalks are found; the submerged mycelium varies from colorless to yellow, orange, or to purplish shades. The color of the heads varies from pale yellowish or slight ochraceous tints through successive intensifications to individual bright yellow almost citrine forms and numerous strains in varying shades of ochraceous, but none in green or umber shades; the heads are globose or radiate; stalks vary greatly in length and diameter but have walls yellow, especially in the outer layer, pitted to rough-pitted as seen with different magnifications, or in occasional dwarf strains with fairly thin walls, the pitting becomes difficult to demonstrate, hence careful examination with a good oil immersion objective is occasionally necessary. Vesicles are globose or occasionally somewhat elliptical, varying greatly in size, with walls usually colorless and much thinner than the stalk wall; sterigmata are always in two series, with primary sterigmata varying as in the *A. niger* group from fairly short to very long, but commonly 15 to 30μ upon Czapek's solution agar, and occasionally much longer on special substrata, secondary sterigmata differ less conspicuously than primary sterigmata, being commonly 7 to 10 by 1.5 to 2.5μ; both series of sterigmata are almost colorless when examined with high magnifications. Conidia are small, sometimes elliptical, but mostly globose or subglobose, smooth or very delicately wrinkled or spinulose without prominent tubercles or bars of color, mostly 2 to 4μ in long axis, larger in occasional strains.

Analysis of group:

1. Conidial heads in fairly pure yellow tints, sulphureus, citrinus or flavus of Saccardo's Chromotaxia, Code de Couleurs 156, 161, 216, 221, or near Ridgway Pl. XV, 15′ and XVI, 23′....*A. sulphureus* series
2. Conidial heads in shades such as cremeus, ochroleucus, ochraceus, or melleus of Saccardo, Code de Couleurs 146, 141, 162, or Ridgway XXX, 19″.....*A. ochraceus* series

Although these two series are closely related, the contrast between the bright yellow series and the buff or ochraceus series is often well-marked in cultures.

A. SULPHUREUS SERIES

Conidial heads in bright yellow tints, not coremiform (Isaria-like) in conidial masses.

Sclerotia not reported.....*A. sulphureus* (Fresenius) Thom and Church
S. ochroleuca Speg.

Sclerotia reported.....*A. quercinus* (Bainier) Thom and Church
S. auricoma Guéguen

Coremiform or Isaria-like.....*A. vitellina* Ridley

S. sulphurea Fresenius, Beitr. zur Mykologie, Heft 3, p. 83, taf. XI, fig. 30–3. 1863. Sylloge 4: 73. 1886.

A description combining information given by Fresenius and that added by Wehmer (Monogr. p. 113) from the examination of Rabenhorst F. Europ. no. 784, recorded as put up by Fresenius follows: Stalks smooth, colorless, up to 13μ, in diameter, up to 1,000μ in length; heads about 150μ (washed in alcohol ?); vesicle up to 90μ; sterigmata in two series, primary sterigmata about twice as long as the secondary, and closely packed to form a radiate head; conidia recorded as globose 2.5 to 3.3μ by Fresenius and as 2.5 by 3.4μ. Sclerotia not mentioned in either description. Not cultivated; not positively identified.

The occurrence of cultures, such as nos. 4660 and 4656, with stalks and heads corresponding to Fresenius' description, and of no. 4754c76, in which the stalks are smooth and colorless at first, suggest that *A. sulphureus* may have been a member of the same group, which is closely related to *A. ochraceus*. The name has been, therefore, applied to the series in the great group with *A. ochraceus*, in which bright yellow heads occur and following Fresenius' description may perhaps be restricted to forms not producing sclerotia; the sclerotium-producing group may perhaps be placed with *S. quercina* Bainier as a sub-series.

S. ochroleuca Speg., Myc. Argent. V, p. 434, in Anal. Mus. Nac. Buenos Aires Ser. 3, t. 13, 1911.

Diagnosis "Caespitulosa, tota ex albo sulfurea, majuscula, hyphis fertilibus erectis continuis, capitulo globosa compactiusculo coronatis; basidia primaria cylindraceo-subclavulata, secundaria etiam subclavulata triplo breviora quaterna v. quina; conidia globosa minuta asperula. Hab. Ad folia et caules Galii relbun loco udo servata, La Plata, Nov. 1910. Obs. Hyphae fertiles saepius 3–8 caespitosae erectae simplices (1 to 2 mm. long by 12 to 14μ crass.) continuae hyalinae, apice abrupte in capitulo vix subpulverulento (250–500μ diam.) plus minusve pallide lutescente expansae; cellula apicalis fertilis globosa (50μ diam.) minutissime densiusculeque papillosa; basidia primaria apice truncata (25 to 30μ by 6μ) secundaria apice rotundata (8 to 10μ by 2μ) omnia hyalina; conidia (4μ diam.) catenulata non v. grosse 1-guttulata. Species plurimis descriptis affinis sed cum nulla bene congruens."

Culture: None.

Until more closely identified this form may be regarded as belonging in the *A. ochraceus* group, although the stalk is specifically described as hyaline. The color and measurements given are more in harmony with *A. sulfureus* than with *A. candidus*, while very pale, if not colorless, stalks are occasionally produced.

A. quercinus (Bainier), syn. *S. quercina* Bainier, Bul. Soc. Bot. France 28:78. 1881, and Sartory, Étude biologique du Sterigmatocystis quer-

cina Bainier, Bul. Soc. Myc. France 26: 349. 1910. Since Sartory was closely associated with Bainier in 1910, the identity of his culture may possibly be accepted and the data from both sources combined.

Colonies with the color of clear oak wood, naples yellow, not ochraceous; stalks up to 10 mm. in length by up to 21μ in diameter, colorless, smooth; heads (?) given as 88.3μ; sterigmata primary and secondary equal in length 10.5μ; conidia globose 4 to 4.2μ smooth; sclerotia yellow up to 0.5 mm., very abundant, especially on carrot where but few stalks develop.

Culture: By Bainier, 1881; by Sartory, 1910; not seen by us.

Smooth stalks as described might separate this form from the *A. ochraceus* group with which its gross appearances suggest relationship; it is automatically reached in the Key from the description as in a series of yellow strains between the white and the black Aspergilli, but is believed really to represent some one of the many sclerotium producing strains of the yellow series of the *A. ochraceus* group represented by Cultures 4754c76, 4186.1, 4078.K-2, which are not identical but certainly belong together as nearly related.

A culture, no. 4640.485, under this name in the Bainier Collection as received in 1922 is a strain of *A. niger*, hence can not be authentic for *S. quercina* as described.

S. auricoma Guéguen, Guéguen,F. Sur une nouvelle espèce de Sterigmatocytis. Bul. Soc. Myc. France 15: 171–187. figs. 1–48. 1899. The describer recognized a close relation of this species to *A. ochraceus*, but describes an "orange wooliness" of the head shown in his figures as the growth of secondary sterigmata into short hyphae instead of spore chains. Normal secondary sterigmata are described in this form as being as long as the primary sterigmata; stalks up to 1 mm. by 10 to 14μ, rough warty, and yellow; vesicles thick-walled up to 50μ diam.; sterigmata, primary 7 to 10.5μ, secondary 7 to 12μ, part of them growing out into yellow hyphae giving an "orange wooliness," hence the name; conidia ovoid, smooth, thick-walled, sulphur yellow to orange 3.5 by 3.8μ. Sclerotia produced on solid media.

Culture: Guéguen.

S. auricoma may be placed in the sclerotia forming section of the series with *A. sulphureus*, but we have not seen it.

S. vitellina Ridley, Jour. Bot. [London] 34: 152, pl. 357, figs. 14–16. 1896. No one has since reported Ridley's organism, which was described from Singapore, as forming coremiform bundles of conidiophores 2 to 3 cm. in height, spreading at the apex and bearing yellow

heads. Significant measurements: Stalks 10 to 12μ in diameter; vesicles 18 to 22μ; primary sterigmata 15 to 18 by 4μ closely packed over whole surface of vesicle; secondary sterigmata 6 to 8μ long; conidia globose 4μ.

This description lacks essential data to place it with certainty in either of the large groups established; the coremia described have been used to establish an arbitrary place for it in the Key. Disregarding the coremia, the organism as described would fit in the yellow series (*A. sulphureus*) of this group. While interlacing masses and ropes of mycelium are occasionally encountered, no Stilbum or Isaria-like coremia have been seen in Aspergillus except as reported by Ridley. Until Ridley's organism is rediscovered and cultivated, it can only be arbitrarily placed among those otherwise apparently similar.

A. OCHRACEUS SERIES

A. ochraceus Wilhelm. (Wilhelm, K. A. Beiträge zur Kenntniss der Pilzgattung Aspergillus. Inaug. Diss. Strassburg, p. 66. 1877). Wilhelm's exsiccati exist as Rabh. F. Europaei no. 2361, from which the identity to a group has been clearly determined by us in the following terms: Stalks several millimeters long by up to 15μ in diameter, with walls 3μ in thickness, rough-pitted, yellow; vesicles 60 to 75μ in diameter; sterigmata in two series, primary often septate and very long, up to 70μ, secondary 10 to 12μ in length; conidia 3.5 by 5μ or 3.5 to 4 or even 4.5μ in diameter, very delicately marked or roughened. In his description of this organism, Wilhelm enumerates characters which are common to a great group of races or strains, world-wide in distribution and, perhaps, represented by some of the synonyms cited by him: *A. ochroleucus* Haller, Enum. Meth., p. 6. 1742; *Monilia ochroleuca* Gmelin, Sys. Nat. II, pt. 2, p. 1487. 1790; *M. sulphurea* Persoon (?), Syn. Meth., p. 691. 1801. *Sterigmatocystis sulphurea* Fresenius, Beitr. zur Myk., p. 83, 1863, was included by Wilhelm, but would be excluded by literal reading of Fresenius' description.

Forms with ochraceous conidial heads and conidia mostly 2.5 to 3.5μ in diameter or more or less elliptical but not exceeding 5μ in long axis. In this series there are many strains with more or less fixed characters. Until separated by careful quantitative studies, the following species may probably be disregarded and cultures "lumped" as A. ochraceus Wilhelm.

S. ochracea (Wilhelm) Schröter. = *A. ochraceus* Wilhelm.

A. melleus Yukawa, Jour. Coll. Agr. Imp. Univ. Tokyo 1, no. 3,

p. 366, taf. 16. 1911. This was described from dried fish (Katsuobushi) in Japan.

Colonies at first colorless, becoming honey color with ripening conidial areas, or brownish yellow in age, stalks 700 to 1,000 by 7 to 25μ, septate, yellow-brown, more or less rough or warty; vesicles 20 to 50μ, subglobose often nodding; sterigmata primary 10 to 22 by 2.5 to 4μ, secondary 10 to 16 by 1 to 2μ; conidia 2.5 to 4μ globose or ellipsoid, smooth. Sclerotia abundant 400 to 700μ in diameter with walls yellowish brown.

Culture and biochemical study: Yukawa.

No. 4291.6 from Hanzawa under this name satisfies the description and puts it in the *A. ochraceus* group.

A. elegans Gasperini, Atti Soc. Toscana Sci. Nat. Pisa, Mem., 8: 328, fasc. 2. 1887; Syn. *S. elegans* (Gasp.) Sacc., Syll. 10: 525; regarded by Gasperini as a probable synonym of *A. ochraceus* Wilhelm; this is probably correct.

Mycelium white; stalks continuous, unbranched, hyaline then pale ochraceous, 1 to 6 mm. long, by 5 to 12μ in diameter, delicately studded with drops; vesicle up to 70μ diameter, radiate, entirely covered with sterigmata; sterigmata, primary 4 to 26μ long, secondary 7 to 14μ long by 1 to 2μ; conidia ochraceous, elliptical to globose up to 3 to 3.5μ, with wall very delicately verruculose, sclerotia not found.

Culture: Gasperini. This organism is not recognized as a separate form by us.

A. alutaceus B. and C. Grevillea 3, no. 25, p. 108. 1875. This (no. 655) was based upon no. 3793 in Curtis' Herbarium now in the Cryptogamic Herbarium of Harvard University. Colonies tan, observed on decaying (*Zea mays*) corn; conidiophores flexuous; conidia globose, alutaceous. No. 3793, from moldy corn, August, 1852, was collected at Society Hill. South Carolina.

A slide made and preserved by Dr. Farlow shows that this species was probably a strain of *A. ochraceus*, since the heads are characteristic in size and in their relation to stalk, and bear sterigmata characteristic in appearance for that species.

Culture: None; this organism is not further determinable, but cited under *A. ochraceus* group.

A. ochraceus var. *microspora* Tiraboschi, Annali di Botanica [Rome] 7: 14. 1908.

This strain was compared with a culture accepted by Ceni as *A. ochraceus* from which it differed in somewhat smaller vesicles, sterig-

mata, and conidia. As noted these differences were: Stalks 500 to 1,500μ by 7 to 12μ, instead of 10 to 15μ; vesicles 30 to 40μ, instead of 40 to 45μ in diameter; primary sterigmata 20 to 32 by 5 to 9μ, instead of 35 to 40 by 7 to 11μ; secondary sterigmata 8 to 10 by 3 to 3.5μ, instead of 8 to 15 by 3 to 3.5μ; conidia 3 to 3.5μ, instead of 4 to 5μ. The growth range reported was minimum 15°C., optimum 20° to 25°C., and maximum 30°C.

The description offered covers one of the smaller strains of this group, but it is very doubtful if that strain could be surely identified.

S. helva Bainier, Bul. Soc. Bot. France 28: 78. 1881. Colonies with the color of lead tannate or "pale cafe au lait"; capitule (? vesicle) 37.8 to 73.5μ; sterigmata, primary 8.4μ, secondary 4.2μ in length; conidia 3.1μ in diameter.

Culture: Bainier. No. 4640.476 from the Bainier Collection is a strain of *A. ochraceus*.

Intermediate species: related to *A. ochraceus* and merging toward the brown forms typified by *A. tamarii*.
Colonies differing from *A. ochraceus* in size of conidia, which are 7 to 8μ in diameter and roughened.....*S. delacroixii*
Conidia somewhat pyriform, showing a delicate tracery of yellow coloring substance.....*A. ostianus*
Conidia, heads, and stalks yellow.....*S. butyracea*

A. delacroixii (Sacc.).

S. delacroixii Sacc., Sylloge 10: 527. Syn. *S. ochracea* Delacroix. The significant data are: Stalks pale yellow, figured by Delacroix as rough, 500 to 1,000μ long; vesicle globose, thick-walled punctate, yellowish; sterigmata, primary 39 by 12μ, secondary from Delacroix's figure about 8 to 10 by 2 to 3μ; conidia globose finely roughened 7 to 8μ.

The diagnostic characters and figures agree with the *A. ochraceus* group, but the conidia are double the usual diameter for members of that group.

S. ochracea Delacroix, Bul. Soc. Myc. France 7: 109, pl. VII, fig. f. 1891. Syn. *S. delacroixii* Sacc. Sylloge 10: 527. q.v. Delacroix described his organism without recognizing the previous use of the specific name.

A. ostianus Wehmer. Ueber einige neue Aspergillus-Arten (A. varians, A. minimus, A. ostianus), Bot. Centralb. 80: 449–461. 1899. Monogr. p. 117, 118, 119, taf. II. no. I.

Colonies, rusty yellow, pale to deep brownish-yellow to cinnamon; stalks up to 2 mm. by about 7μ, figured as rough; vesicles globose, 35 to 40μ diam.; sterigmata, primary 35 by 8μ, secondary 13 by 5μ, mostly in one series however; conidia slightly elliptical to globose, mostly smooth, yellowish 4 to 5μ.

Culture no. 4724.35 from Raistrick under this name varies from this description in our cultures: Stalks about 1,000μ in length by 10μ in diameter, yellow in outer layers of the cell-wall, densely pitted, also warty with concretions; vesicles about 40μ in diameter, with walls yellow and pitted, brittle, hence easily crushed; primary sterigmata 15 to 20μ long; conidia somewhat pyriform as in *A. flavus* about 5μ in long axis by 3 to 4μ, or in age 6μ by 4.5μ with delicate color bars arranged nearly as the pitted markings of *A. flavus;* sclerotia produced.

Culture: Wehmer, probably correctly represented by *no. 4724.35.* Not thus far found in America.

S. butyracea Bainier, Bull. Soc. Bot. France 27: 29. 1880. C. Roumeguère's Fungi Gallici Exsiccati, no. 995 is recorded as Bainier's material.

Examination of the specimen shows a black Aspergillus with conidia 6 to 6.8μ in diam., probably Bainier's *S. fusca* with an admixture of a yellow species, corresponding more or less closely to Bainier's description: Colonies butter yellow, including stalks, heads, and conidia; stalks yellow, in mounts finely punctate or pitted, 13 to 16μ in diameter; sterigmata primary up to 25μ (in mounts up to 16μ), secondary 10 to 12μ in length; conidia described as smooth 5.2μ, but those found in the material were rough and up to 6.3μ.

Culture: Bainier; not found by us; yellow stalks and conidia smooth or nearly so, ally it closely to *A. ochraceus;* if the conidia are correctly determined as rough, it approaches more nearly to *A. ostianus* Wehmer.

Stalks with walls colorless (or only slightly colored at apex) and pitted.
Conidia roughened with spinules, tubercles or bars of coloring substance (compare p. 184).
Sterigmata in 1 series (compare p. 198)
Conidia golden yellow when young, brown in age, 5 to 9 by 5 to 6μ *A. citrisporus* von Höhnel
Conidia umbrinus..... *A. terricola* Marchal
A. terricola var. *americana* Marchal
A. lutescens Bainier

A. citrisporus von Höhnel. Fragmente zur Mykologie (I. Mittheilung). Sitzungsber. K. Akad. Wiss. Wien, Math.-Naturw. Kl. III, 1

Abt., p. 987. 1902. This form was described by von Höhnel from excrement of larvae. A specimen was collected by Peck at Sandlake, N. Y. from excrement of caterpillars and preserved in the herbarium of the New York Botanical Garden. Another specimen of this species is found in the collection of the Michigan Academy of Science as determined by Kauffman and seen by us.

Cultural description from Thom and Church, Am. Jour. Bot. 8: 119. 1921, based upon no 4186.10: Colonies upon Czapek's solution agar with colorless submerged mycelium; conidial areas at first yellow, then golden, and finally fulvous; stalks 1 to 2 mm. high, up to 20 to 25μ in diameter, turgid when young, often collapsing in age or when exposed to dry air, septate, with walls thin (1μ or less mostly), appearing to be studded with fine granules when examined directly (in air) but appearing smooth in liquid except when high magnification and great care are used to demonstrate the abundant pitting; with heads up to 500μ in diameter; vesicles 30 to 50μ in diameter, nearly globose, and fertile over nearly the entire surface; sterigmata in one series, 8 to 12μ by 3 to 4μ with long, loosely radiating conidial chains. Conidia yellow or golden, then brown, lemon-shaped, 5 to 9 by 5 to 6μ, rough from irregularly branching ridges of yellow to brown coloring matter between the inner and outer wall. Sclerotia are occasionally found. Cultures from excrement of caterpillars by Dr. Roland Thaxter, no. 4186.10, who reports perithecia (personal communication) but we have failed thus far to obtain mature asci for description

A. terricola Marchal. (Marchal, Émile) Sur une espèce nouvelle du genre Aspergillus Micheli; *A. terricola.* In Rev. Mycologique 15, no. 59, p. 101–103. 1893. Possible synonym *A. lutescens* Bainier, nomen nudum, undescribed culture in collection (no. 4640.478).

Colonies "terre d'ombre," umbrinus; mycelial hyphae 3 to 5μ diam., without anastomoses; stalks hyaline continuous or septate in age, 7 to 10μ by 600 to 1,000μ (whole depth of colony growth); vesicle subglobose hyaline 30 to 50μ, radiately covered with sterigmata; sterigmata 12 to 15 by 4 to 7μ; conidia umber (Sacc.), ovate or elliptical then globose, rough, with colorless connective.

Culture: By Marchal from soil in Belgium; not reported elsewhere, see variety.

A. terricola var. *americana* Marchal, cultural description Thom and Church, Am. Jour. Bot. 8: 125. 1921; compare Plate III. 4838. Colonies on Czapek's solution agar from shades near yellow ocher (Ridgway XV, 17′) when young to Dresden brown or mummy brown

of the same plate (near Saccardo's umbrinus); aerial growth consisting of crowded conidiophores, stalks 300 to 600μ by 6 to 8μ, walls pitted; heads radiate; vesicles up to 20μ in diameter; sterigmata in one series, 7 to 10μ by 2 to 4μ; conidia tuberculate from the presence of color bars variously distributed between the outer and inner wall, ovate, from 3 by 5μ up to 5 by 7μ or nearly globose, usually about 5.5μ, occasionally 5 to 8μ in diameter. Culture by F. M. Scales from Georgia soil.

The type culture was isolated by Scales from redland soil in Georgia and discussed by Scales, F. M. The enzymes of Aspergillus terricola. Jour. Biol. Chem. 19: 459–472. 1914, under the name *A. terricola* Marchal. Scales' culture was submitted to Marchal, who designated the form as *A. terricola* var. *americana* Marchal, distinguished as follows: *A. terricola* var. *americana* Marchal. "The dimensions of the vesicles 14 to 20 instead of 30 to 50μ, of the sterigmata 5.6 to 10.5 by 2.2μ instead of 12 to 15 by 4 to 7μ; the spores only very delicately verrucose, separate your fungus from *A. terricola.*"

A. lutescens, name on test tube (our no. 4640.478) of the Bainier Collection. Probably *A. terricola* Marchal. Description from culture 4640.478 in Czapek's solution agar:

Colonies floccose-wooly, white over deep areas of agar, becoming rusty yellow as conidia formation begins and develops unevenly over the surface, and finally with conidial areas chestnut brown; reverse of colony pale yellow; stalks 12 to 15μ in diameter, varying greatly in length, pitting present but not conspicuous, not giving a rough appearance; vesicles 20 to 40μ in diameter; heads radiate or globose; sterigmata, primary about 8 by 5μ, secondary about 10 by 5μ; conidia from 5 by 7μ to subglobose 8 to 9μ, rough with tubercles of color.

Culture: Bainier Collection no. 4640.478, no description found; to be dropped.

THE A. TAMARII GROUP

Sterigmata in either 1 or in 2 series in the same colony and often in the same head.....*A. tamarii* group

Colonies orange yellow at first then deep brown; conidia pyriform to globose 5 to 8μ in long axis, roughened with tubercles and bars of color.....*A. tamarii* Kita.

Possible synonym.....*A. fulvus* Mont.

Probable synonyms.....*A. spadix* Amons

A. umbrinus Patterson

Colonies pale brick red.....*A. gigas* Speg.

A. tamarii Kita. (Kita, G., Einige japanische Schimmelpilze, in Centralb. f. Bakt. etc., 2 Abt., 37, no. 17/21, pp. 433–452. 1913). Characterization of *A. tamarii* Kita, (from our culture no. 4235I2, identified by Kita) see Thom and Church Am. Jour. Bot. 8: 118. 1921; Compare Plate II, 4235I2.

Colonies on Czapek's solution agar with cane sugar spreading broadly, with vegetative hyphae mostly submerged, with fruiting areas at first colorless, then passing through orange-yellow shades to brown in old colonies (variously Isabella color, light brownish olive, buffy citrine, medal bronze, or raw umber. Ridgway, column 19, on Plates XXX, XVI, IV, and column 17, Plate III), not showing true green; reverse uncolored or occasionally pinkish; stalks arising from submerged hyphae, up to 1 to 2 mm. in length, becoming several millimeters in length upon corn or other concentrated media, 10 to 20μ in diameter, increasing in diameter toward the apex and passing rather abruptly into vesicles, with walls rather thick, 1 to 2μ, becoming abruptly thinner at the base of the vesicle, pitted more prominently in upper than lower half (often appearing as rough or echinulate with low magnifications) and frequently showing irregular thickenings within; vesicles 25 to 50μ in diameter with fairly thin walls which frequently crush in mounts; heads varying greatly in size in the same fruiting area, from more or less columnar to nearly but not completely globose and up to 350μ in diameter, with radiating chains and columns of conidia; sterigmata, one series in small heads, two series in large heads, primary commonly 7 to 10 by 3 to 4μ, becoming 20 to 35μ long in gigantic heads upon corn, secondary 7 to 10 by 3μ; conidia more or less pyriform toward globose, tuberculate especially at the distal end in the chain, 5, 6, occasionally up to 8μ in diameter, rough from prominent masses and bars of orange-yellow coloring matter deposited under the loose outer wall upon the firm inner wall. Sclerotia occasionally produced, usually purple or reddish purple, globose to pyriform with apex white.

The organism of Kita (recognized by him in our no. 4235I2) proves to have been one of a great group represented in our collection and in series examined by many strains from North and South America, from Japan, China, India, and a very few from Europe. The outstanding characters are orange-yellow to brown colonies; stalks coarse, pitted, sometimes with pitting obscure, colorless; vesicles large radiate; sterigmata in one or two series but usually with single and double series in the same head; conidia more or less pyriform 5 to 8μ in long axis with tubercles or bars of orange-yellow coloring matter between

the inner and outer cell-walls. Sclerotia are occasionally found, but perithecia are not reported.

Culture: Kita, Thom and Church.

Race 3565 in Amer. Journ. Bot. 5: 88. 1918 was incorrectly referred to *A. castaneus* instead of to *A. tamarii.*

A. fulvus Montagne, Plantes Cellulaires, Centurie VI, no. 82, in Ann. Sci. Nat. Bot. sér. 3, 12, p. 298. 1849; Syn. *S. fulva* Sacc. Syll. 4: 74.

Described from diseased silkworms in southern France, as orange drying fulvous; with stalks up to 2 mm. long by up to 20μ in diameter, delicately roughened; heads globose about 150μ in diameter, radiate; sterigmata up to 50μ long "multifid at the apex," hence in 2 series; conidia hyaline, globose, with endospore and epispore blended (confuso).

Culture: None. This organism has not been seen by us and its exact affinities must remain unsettled, but its stalks are reported as roughened and the colors described might easily place it not far from *A. tamarii.*

A. spadix Amons. (Amons, W. J. Th. Bijdrage tot de Kennis van de fora van Achteruitgaande Suiker. Archief voor de Suikerindustrie in Nederlandsch-Indië Jaarg. 29, Deel 1, pp. 12–14. Jan.–June, 1921. From the description, this is a synonym of *A. tamarii* Kita. q.v.

Colonies yellow brown to deep brown growing well on common laboratory media, without aerial mycelium; in reverse colorless; rice colored light violet at first then light fuscous brown; stalks up to 2 to 3 mm. by 8 to 9μ with walls about 0.9μ and pitted or rough; vesicles globose up to 50μ in diameter or almost clavate in small heads; conidia 5.5 to 7.2μ rough.

Culture: Amons.

This species was found occasionally in spoiling sugar in the Dutch East Indies by Amons, but is clearly *A. tamarii* as described by Kita and in our collection from many parts of the world, including Singapore.

A. umbrinus Patterson (Patterson, Flora W. Bull. Torrey Bot. Club 27: 284. 1900). The original material appears to be lost. It was isolated in 1900 by Dr. E. A. Bessey from a moldy Brazil nut. He has recently (1924) obtained a culture apparently similar to the original also from a Brazil nut. This culture, no. 4735, is certainly a strain of the group designated here as *A. tamarii.* Mrs. Patterson's description lacks the details to establish identity, hence not supported

by exsiccati had, perhaps, better be dropped, although Dr. Bessey's recent culture establishes a presumption of identity.

A. cacao, nomen nodum. This name has appeared upon a culture in the Bainier Collection (no. 4640.397) and has been contributed also by Pribram (no. 4777.4). The organism is a strain of *A. tamarii.*

A gigas Speg., Myc. Arg. V, p. 434. In An. Mus. Nac. Buenos Aires ser. 3, t. 13, 1911.

Diagnosis "Caespitosus maximus testaceus, hyphis hyalinis continuis, capitulo globoso compactiusculo; conidia minuta laevia pallide isabellina. Hab. Ad folia subputrescentia Coffeae arabicae diu loca udo servata. La Plata, Nov. 1910. Obs. Hyphae fertiles erectae rigidulae 2-5-caespitosae (2–3 mm. long. = 20μ crass.), simplices, apice abrupte in vesciculam globosam (120μ diam.) pallidissime rufescentem dense minuteque papillosam productae; conidia longissime catenulata, globosa (5–6μ diam.) eguttulata laevia v. subtillime imperspicueque colliculosa, capitulum sphaeroideum maximum (0,75–1 mm. diam.) efficientia. Species statura vere abnormis mox dignoscenda."

Culture: None.

Arbitrarily placed in group with *A. tamarii,* but a possible mutant of *A. niger* from the description.

Conidia pitted rather than roughened with color bars and tubercles
Gigantic forms.
With aspect of *A. ochraceus*.....*A. penicillopsis* Hennings
With heads red or reddish.....*A. erythrocephalus* B. & C. n.n.

A. penicillopsis (Henngs.) Racib. P. Hennings as *Stilbothamnium penicillopsis* P. Henn. & E. Nym. described in Fungi Monsunenses (Warburg, O. Monsunia, Bd. I, p. 37, 1900. Leipzig); exsiccati of type in Pathological Collections, U. S. Dept. Agr., Bureau of Plant Industry, as: Raciborski no. 87, in Crypt. Parasiticae Java.

This material shows an Aspergillus with the color and general appearance of *A. ochraceus* but of gigantic proportions. Measurements as follows: Stalk 50 to 70μ in diameter, 10 mm. or more long with walls 7 to 12μ thick. Surface ragged and more or less pitted. Vesicle up to 175μ in diameter with walls 7μ thick, marked with a deep pit for each sterigma. Sterigmata primary 50 to 90μ, at times 120 by 8 to 10μ at the outer end. Sometimes with one cross wall. Secondary 15 to 25μ by 3 to 4μ, clustered on the apex of the primary. Occasionally with a sterile cell interposed between secondaries and primaries. Conidia 8 to 12 by 5 to 8μ, elliptical, pitted, yellowish.

Type material alone known.

A. erythrocephalus B. & C. (Berkeley, M. J., and Curtis, M. A., Jour. Linn. Soc. [London], Bot. 10: 362. 1869. See Fungi Cubensis Wrightiana, 1868, Type no. 642 in Curtis Herbarium deposited in the Cryptogamic Herbarium of Harvard University, bears Wright's no. 764.) Part of the original material was removed by Dr. Farlow and given to us for study.

Microscopic examination of this type specimen gives measurements as follows: Stalks 45 to 70μ in diameter, up to 2 mm. in length, with walls very heavy 5 to 12μ thick, varying from 5 to 6μ in broader part to 10 to 12μ at narrower base, pitted or roughened; vesicle up to 100μ in diameter, nearly globose, fertile all over; head washed free from spores about 150μ diameter; sterigmata, primary 8 to 10μ in length, secondary 8 to 9μ in length; conidia 8 by 6μ, commonly varying, 8 to 12 by 5 to 9μ, finely pitted or roughened with rather thin walls. Colors in the material are questionable on account of the age of the collection.

Cultures: None. Type material only known. Placed between the *A. tamarii* and *A. flavus* groups. The emended description is offered on account of the existence of the type specimen with very conspicuous characters under a name only very briefly described in 1869.

SECTION XII

The Yellow-green Aspergilli, A. flavus-oryzae

Summary of group characters:

Colonies mostly some shade of green or yellow green or greenish yellow. Some strains lose the green color in age; some appear to lack green color but to have the same underlying shades of yellow as in the group. Conidia without color bars sometimes almost smooth but mostly marked with more or less winding pits giving frequently a falsely rough appearance when studied with low magnifications. No separation of primary (outer) from secondary (inner) conidial wall is visible.

This group contains a great series of races, strains or species represented conspicuously in the literature by *A. flavus* Link and *A. oryzae* (Ahlburg) Cohn. If the organism designated *A. flavus* by Brefeld and Wehmer (our no. 108) is compared with *A. oryzae* as distributed for many years (no. 113 of our series) the contrast is great. If on the other hand, a whole series of organisms occurring in nature and especially including those utilized in the soy and saké fermentations is brought together and the members compared, this distinctness disappears. The following characterizations of these forms as typifying sections of this great group are presented with subsequent data showing transition forms bridging the gap between them practically completely.

A. oryzae (Ahlburg) Cohn, (Cohn, F. Ueber Schimmelpilze als Gärungserreger. Jahresb. Schles. Gesell. Vaterl. Cultur (1883) 61: 226. Breslau, 1884). Syn. *Eurotium oryzae* Ahlb. The name *E. oryzae* with an incomplete description for the saké organism was published by Korschelt (Korschelt, O. Ueber Saké, das alkoholische Getränk der Japaner. Dingler's Polytechnisches Jour. 230: 330. 1878) as taken from a letter from "Herr Ahlburg."

The following characterization from culture (no. 113) is taken from Thom and Church, Amer. Jour. Bot. 8: 106. 1921: Colonies on Czapek's solution agar spreading broadly, pale greenish yellow (at its greenest about lime green to mignonette green. Ridgway XXXI, 25″ YG-Y), with the green fading early to leave yellowish brown shades; sclerotia dark, produced occasionally, few and in clumps; mycelium and agar uncolored; stalks 2 to several millimeters long, up to 20 to 25μ in

diameter; heads both large and small in the same culture, predominantly large, globose, and radiate rather than calyptrate; sterigmata most commonly in 1-series, occasionally in 2-series, primary sterigmata up to 8 to 12 by 5μ, secondary when present 7 to 10 by 3μ; conidia pyriform, colorless to very slightly yellow, with walls so thickened as to leave circular, elongated, or winding pits giving a rough or echinulate effect when viewed with low magnification, varying in size, predominantly larger than *A. flavus*, 3 by 4μ, 4 by 5μ, 5 by 6μ, 6 by 7μ, occasionally 5 to 6 by 8 to 10μ.

Zikes (125) reported perithecium formation in this species, but his culture sent to us (our no. 4627) proved to be a member of the *A. glaucus* group. Bezssonof (126) also reports perithecia but without data to insure discrimination between perithecia and sclerotia.

Many strains closely resembling no. 113 have since been examined, together with forms manifestly related but diverging in shades of color, in measurements and in effects upon substrata. This diagnostic description will serve to locate an organism to the series or group, but will not identify a particular strain.

Costantin and Lucet's variety *basidiferens* is added here as a synonym described by them on the assumption that the appearance of two series of sterigmata was a separating character.

A. oryzae var. *basidiferens* Costantin and Lucet. Ann. Sci. Nat. Bot. (IX) 2: 167. 1905. The describers found both primary and secondary sterigmata upon a culture received by them as *A. oryzae*. Without cultivating any other strain, they describe this form as a new variety. Although double sterigmata are not mentioned in Wehmer's description, all strains seen by us have double sterigmata, at least, under some conditions of culture. Hence the varietal name should be dropped. This varietal name is incorrectly cited by Saccardo in the Sylloge 22 as var. *basidifer*.

A. flavus: Characterization of *A. flavus* series from Thom and Church, Am. Jour. Bot. 8: 105. 1921; compare Plate II, 108. Colonies on Czapek's solution agar with sucrose, spreading widely, with floccosity limited to scanty growth of a few aerial hyphae in older and dryer areas among the erect crowded conidiophores; sclerotia at first white, then brown, hard, parenchymatous, in a few strains white-tipped, produced abundantly by some strains, scantily by others under undefined conditions, not or rarely by still others; perithecia not found. Conidial areas ranging in color in different races from sea-foam yellow through chartreuse yellow, citron green or lime green, to mignonette green,

Krönberg's green, or more rarely to ivy green (see Ridgway XXXI, 25″ f, d, b, i, k, m; approximately C. D. C. 270, 271, 266, 252, 253, 257), persistent or changing in very old colonies toward Isabella color to brownish olive (Ridgway XXX, 19″ i, m), zinc orange (Ridgway XV, 13′), or even Saccardo's umber (Ridgway XXIX, 17″ k); reverse (or under side) and agar either uncolored or more or less intensely yellowed, from pinkish buff, cinnamon buff, to clay or even Saccardo's umber (Ridgway XXIX, 17″ d, b, to k), or in some cases even darker brown in old and dry cultures. Stalks arising separately from substratum, 400 to 700μ, even to 1000μ long, 5 to 15μ in diameter, broadening upward, with walls colorless, so pitted as to appear rough or spiny with low magnification, occasionally granuliferous, varying in thickness, gradually enlarging to form a vesicle 10 to 30 or even 40μ in diameter. Heads in every colony varying from small with a few chains of conidia to very large stellate or columnar masses, or both mixed in the same area; small heads with small domelike vesicles and a single series of a few sterigmata up to 10 to 15μ by 3 to 5μ; larger heads partly with simple sterigmata, partly with branched or double series, or with both in the same head; primary sterigmata 7 to 10μ by 3 to 4μ; secondary series 7 to 10μ by 2.5 to 3.5μ; conidia pyriform to almost globose, from almost colorless to yellow-green, sometimes almost smooth, mostly with walls so thickened as to leave circular, elongated, or winding pits, giving a rough or echinulate effect when viewed with low magnification, varying in size in different strains and even in the same culture, frequently 2 by 3μ, 3 by 4μ, 4 by 5μ, or 5 by 6μ in diameter, or even larger in some strains.

Dangeard (Le Botaniste 10: 154–158. 1907) reports the sclerotia as finally developing an ascogenous phase, but this has not been confirmed and a study of Dangeard's figures leads us to doubt if his organism even belonged to this group.

Organisms falling in the aggregate species *A. flavus* as thus characterized are exceedingly common in the routine culture work of many laboratories. They occur upon cereal grains as contaminations when the grains are sound, as causes of decay when spoilage is evident. They are abundant in mill products and thus are carried into the bakeshop to appear as molds of bread and other bakery products. In the cultural examination of soil and all soil contaminated products, *A. flavus* colonies constantly appear. Molds of this group are common agents of decay in materials containing starch, sugar, and related products, but are not found commonly on meats, although *A. gymnosardae* has been described from fermenting fish.

Grain molded with strains of *A. flavus* has been charged with the poisoning of cattle and hogs in several instances, but experiments in which bacteria were excluded have thus far proved non-toxic. Whether poisonous by-products of decomposition are occasionally produced remains, therefore, uncertain.

Members of the group have been occasionally found in pathological lesions and when tested on experimental animals certain species were reported to be pathogenic, but natural infection by such organisms is certainly rare in mammals. In insects, on the other hand, Speare (see *A. parasiticus*) has found one of them destroying the mealy bug of sugar cane in Hawaii. Johnston sent the same type of organism upon dead and dying mealy bugs from cane in the West Indies, but showed later that various members of the group were able to infect these insects. Another member of the series has recently been contributed as attacking the European corn borer in New Jersey. They have also been reported (Vincens, loc. cit.) as destroying bees both by European investigators and in experiments in the U. S. Bureau of Entomology (personal communication of Burnside).

The *A. oryzae* series, on the other hand, is not so fully represented among miscellaneous cultures from widely different products and widely separated regions. A very few members of this series have been found from sources apparently not connected with the oriental fermentation industry; for example, one culture from a moldy Brazil nut. When, however, the cultures used by the Japanese students of saké and soy fermentation problems are studied, the separateness of these two series in the group breaks down. A large collection of these organisms has been brought together by courtesy of Kita, T. Takahashi, Hanzawa, Saito, and Oshima, together with materials collected for the Bureau of Chemistry by Dr. Kin in China, and our own isolations from materials from the Orient and from the commercial preparations of Takamine. Examination of this material, shows that in the usage of the workers in the fermentation industry of Japan, the name *A. oryzae* is applied indefinitely to the whole group, although as indicated in a study (127) of Takahashi's series of cultures most of these forms approach more closely to the *A. oryzae* section of the whole group than to the *A. flavus* section.

In Takahashi's report these are lettered[1] with the alphabet from A to P, then skip to Z, and all are regarded as *A. oryzae*.

[1] The lettering is maintained to correspond with Dr. Takahashi's usage in his own papers. Takahashi, T., and Yamamota, T. On the physiological differences of the varieties of Aspergillus oryzae employed in the three main industries in Japan, namely, saké, shoyu, and tamari manufacture. Jour. Col. Agr. Imp. Univ. Tokyo 5: 153–161. 1913.

These cultures were transferred and grown under conditions as uniform as possible in Czapek's solution agar. The resulting colonies were arranged into a series to correspond with our conception of the relationships involved. This may be tabulated as follows:

Takahashi strains arranged in order of appearance of colonies:

H. White, nearly sterile floccose mycelium.
O. Slight fruiting, predominantly yellow.
B. Increase of fruiting, still a floccose colony.
G. Further development of fruit at expense of floccosity.
Z. Long stalks, large heads, floccose effect.
D. Mycelium and long-stalked fruits, both evident.
N. Abundant stalks and heads, no green color.
F. Close resemblance to no. 113, *A. oryzae* of Wehmer.
I. Short-stalked, form otherwise near no. 113.
A. Still shorter.
M. Same morphology, green color more prominent.
L. Slightly paler form with shorter stalks.
C. Close to no. 108, *A. flavus* of Brefeld and Wehmer.
P. Shorter stalks (crowded; more slender type), green passing to reddish brown, suggests the description of *A. micro-virido-citrinus*.
J, K, E. Aberrant forms suggesting the same line of transformation from strain C as is found in *A. effusus*, though differing from previously examined representatives.
Similarly Z, G, B, O, and H are progressive reductions from the *A. oryzae* type found in strain F.

This table shows strain F to represent approximately the form already described as *A. oryzae* (no. 113). With almost entire loss of green color and progressively increasing floccosity, strains N, D, G, B, O, and H end at H in almost complete loss of conidium production. The absence of all green color makes strain N conspicuous as a variation which might easily be regarded as a distinct species. From F the strains I, A, M, and L grade in appearance toward C, which is closely similar to *A. flavus* as already described (no. 108).

The tendency toward floccosity and toward quick disappearance of green color appears again in L.

P is a more slender, delicate form than C, with crowded stalks, and also loses its color quickly.

The species discussed in the following paragraphs are either synonyms of *A. flavus* or represent strains more or less characteristic of sections of the group: Identification of any particular culture as solely correctly representing any one of these forms is probably impossible.

Species described as having sterigmata in 1 series. . *A. oryzae*, already discussed
A. parasiticus Speare
A. flavus forma *maydis* Ciferri
Species commonly showing simple and double sterigmata in the same culture and even in the same head..... *A. flavus* series

A. parasiticus Speare, Hawaiian Sugar Planters' Exp. Sta., Path. & Physiol. Ser. Bul. 12, p. 38, pl. 3–4. 1912. Speare, working in Honolulu, found one of the *A. flavus* group parasitic upon the mealy bug of sugar cane (*Pseudococcus calceolariae* Mask), and described his strain as *A. parasiticus*. A culture with the same characters was isolated by one of us from mealy bugs obtained from Demerara; another culture was isolated from cane sugar in New Orleans by Kopeloff. However, other strains of the *flavus* group were isolated by Johnston from mealy bugs in Porto Rico; reinfection experiments by Johnston, while not conclusive, established a presumption of infectiousness as a strain or race character unconnected with the specific morphology of Speare's *A. parasiticus*.

Speare's organism when grown on Czapek's solution agar differs from the commoner forms of *A. flavus* in its predominantly greener color (near ivy green Ridgway XXXI, 25″ m), in short stalks usually 200 to 400μ long, in heads with usually a single series of sterigmata 7 to 10μ long by 3 to 5μ. No sclerotia have been seen. The mycelium is uncolored. Otherwise the characters are those of the *A. flavus* group.

Culture: Speare; type no. 3509 received from Speare.

A. flavus forma *maydis* Ciferri. (Ciferri, R. Contrib. allo Studio dei Mic. del Mais, in Bul. Soc. Bot. It., no. 7, p. 75. 1921; also Ann. Mycol. 20: 46. 1922.) Described from rotting grains of maize: differing from the species in stalks 700 to 1000μ in length; vesicles 85μ in diameter; sterigmata 7 to 14 by 2.5μ and conidia smooth, yellowish-hyaline 2.5 to 3μ.

Culture: Not reported.

This form has not been seen but strains in this group occasionally show conidia almost as small as these with more or less complete suppression of green color.

A. FLAVUS SERIES

In addition to *A. flavus* Link represented by the organism of Wehmer and Brefeld and described already in characterizations of the group, the following names represent synonyms and forms typifying sections of the series such as *A. pseudoflavus* and *A. effusus* or forms found associated with a particular product as *A. gymnosardae*.

The questionable floccose series represented by *A. effusus* Tiraboschi as interpreted to account for a series of cultures is added as related to *A. flavus*.

A. flavus Link, Obs. p. 16. 1809. "A. flavus, caespitibus laxis, floccis albis erectis, capitulis junioribus albis, adultioribus flavis. Frequens in plantis siccis herbariorum." Cited by Link in Sp. Plant. 6: 66. 1824, as synonym of *Monilia flava* Persoon, Myc. 1, p. 30. De Bary and Woronin in describing *Eurotium Aspergillus flavus* (De Bary, A., and Woronin, M., Beiträge zur Morphologie und Physiologie der Pilze, III Reihe, p. 380, 1870) believed their material to be identical with the species of Link. A culture by Brefeld preserved in Rabenhorst, Fungi Europaei Edit. Nov. ser. II, no. 2135, is cited by Wilhelm (Wilhelm, K. A. Beiträge zur Kenntniss der Pilzgattung Aspergillus. Inaug. Diss. Strassburg, p. 59. 1877), and later by Wehmer Monogr. p. 81. The continuity of the usage of the name *A. flavus* seems, therefore, well established. We have examined a packet of this material in the collection of the New York Botanical Garden, which is certainly the organism we have described as *A. flavus*. Costantin and Lucet (loc. cit., pp. 162, 163) attribute the name *A. flavus* incorrectly to Wilhelm. We have further searched in moldy herbarium material for the organism commonly known under this name. In a packet collected by Weir in 1924 in Brazil, heads truly "flavus" in color were found and transferred to culture media producing colonies of the well-known yellow-green form as understood by Brefeld, Wilhelm, and Wehmer.

A. wehmeri Costantin and Lucet. Ann. Sci. Nat. Bot. (IX) 2: 162. 1905. The name is proposed by Costantin and Lucet for the organism which Brefeld and Wehmer described as *A. flavus* Link and which is so used in this paper. The uncertainties in the identification of Link's species do not seem important enough to justify the change of name. *A. wehmeri* is to be regarded as a synonym of *A. flavus*.

A. variabilis Gasperini (Gasperini, G. Atti Soc. Toscana Nat. Sci. Pisa, Mem. 8, fasc. 2: 326. 1887). From the description, this was probably some strain of the *A. flavus* group. Both large and small heads were found in the same colony; the sterigmata were simple or double; the conidia have the range of form and size characteristic of the group. Gasperini does not appear to have known *A. flavus*.

A. gymnosardae Yukawa. Jour. Col. Agr. Imp. Univ. Tokyo I: 362, pl. 18, figs. 1–7. 1911. This fungus was found by Yukawa under the name "awokabi" and is described by him as essential to the ripening of the tuna fish preparation, "katsuobushi."

The dimensions given are intermediate between those of *A. flavus* and of *A. oryzae*, and closely approximate those of *A. pseudoflavus*. Although we have cultures related to these forms, we have not been able to identify these intermediates except as members of the *A. flavus-oryzae* group. As described: Colonies white to yellowish green to leaf green, becoming dark dirty brown in age; stalks 1 to 2.5 mm. in length by 10 to 25μ in diam., more or less rough; vesicles 20 to 40μ in diam.; sterigmata either in single series 12 to 25 by 5 to 6μ or with primaries 10 to 20 by 5 to 6μ and secondaries 10 by 2 to 3μ; conidia 4 to 6μ in long axis. Sclerotia not found.

Culture and biochemical study: Yukawa.

A. pseudoflavus Saito. Centralb. Bakt., etc., 2 Abt., 18, no. 1/3, p. 34, figs. 15–18. 1907; syn. *S. pseudo-flava* (Saito) Sacc. Syll. 22: 1260. 1913.

A form with stalks 1 to 2 mm. long, otherwise with the characters of *A. flavus;* Saito separates it because primary and secondary sterigmata are found as well as simple sterigmata. Since this observation applies to a large number of forms in the group, there is little basis for maintaining this name.

Culture: By Saito; not identified by us; in the long stalked section of *A. flavus* group.

A. nölting Hallier, Zeitschr. Parasit. (not found) cited by Cattaneo and Oliva in Arch. Lab. Bot. Critt. Garovaglio 5: 122. 1888. The conidia are described as yellowish. Other data are lacking but *A. flavus* alone of the organisms reported from the human ear commonly shows yellowish conidia.

Culture: None; to be dropped.

A. micro-virido-citrinus Costantin and Lucet, Ann. Sci. Nat. Bot. (IX) 2: 158. 1905. The appearances of colonies and measurements of stalks, heads, and spores indicate a form intermediate between *A. flavus* and *A. oryzae* except for its small conidia. The description is very nearly satisfied by Takahashi's culture P. It was found to grow between 15° and 45°C. and to be pathogenic to rabbits.

Colonies were greenish yellow, predominantly yellow, but containing some definitely green admixture in contrast to *A. oryzae*, which often lacks green color entirely; stalks 600 to 1700μ in length, up to 21μ in diameter near the vesicle, uncolored, "granular" (= pitted) above, smooth toward the base; vesicles 24 to 62μ in diameter; sterigmata varying in size and arrangement with the size of the heads examined; conidia globose, smooth 3 to 4.6μ (3.1 as a minimum to occasional diameters of 5.5μ).

Culture and biochemical study: Costantin and Lucet.

A. siebenmanni Costantin and Lucet. Ann. Sci. Nat. Bot. (IX) 2: 162. 1905. This name is based upon Siebenmann's description of an organism from the human ear identified by Siebenmann as *A. flavus*, but regarded by the describers from the description given by Siebenmann as a separate species. (See Siebenmann, F. Die Fadenpilze Aspergillus flavus, nige ru. fumigatus; Eurotium repens und ihre Beziehungen zu Otomycosis aspergillina. Zeitschr. f. Ohrenheilk. 12. 1883. Die Schimmelmycosen des menschlichen Ohres. Wiesbaden, 1889.) The data given place the organism correctly in the *A. flavus* group, but are not complete enough to separate it.

A. novus nomen nudum, see Wehmer, C. Ueber die Verflüssigung der Gelatine durch Pilze. Chemiker-Zeitung 19: 2038. 1895. He lists *A. novus* among Aspergilli used in experimental work; no description is given. The same article is also cited by Wehmer in Lafar, Handb. d. Techn. Myk. 2 Aufl., Bd. 4, Lieferung 11, p. 255, without additional data. In his monograph (loc. cit., p. 90), however, Wehmer omits all reference to the name. A culture under this name (no. 141) was received from the "Centraalstelle" in Amsterdam in 1910, and proved to be a strain of *A. flavus*. Apparently the same strain was again received under the name from Raistrick (no. 4724.46) in 1924. No justification for maintaining this name appears upon either bibliographic or morphological grounds.

A. effusus Tiraboschi, Ann. di Bot. [Rome] 7: 16, fasc. 1. 1908. See also Thom and Church, Am. Jour. Bot. 8: 109–110. 1921. Described originally from rotten corn (*Zea Mays*); belongs to the *A. flavus-oryzae* group; compare Plate II, 130.

Characterized by Thom and Church: Colonies on Czapek's solution agar with sucrose, broadly spreading, effused floccose, or cottony, white becoming slowly dirty yellowish or in restricted areas greenish yellow; reverse and agar yellowish. Stalks either *A. flavus*-like arising directly from the substratum, up to 500μ long and frequently with large radiate heads, or predominantly in the form of branching, trailing, thick-walled hyphae, each segment consisting of a long, thick-walled, fertile cell bearing a perpendicular branch (stalk) usually less than 100μ long by 5 to 10μ in diameter, with walls pitted and sometimes granuliferous, bearing usually columnar heads; vesicles in small heads up to 20μ in diameter, occasionally larger, sterigmata in one series, 6 to 10μ by 3 to 5μ, mostly on apex only of vesicle; larger heads with either simple or branched sterigmata as in *A. flavus*. Conidia pitted as in *A. flavus*,

pale yellow, rather thin-walled, pyriform to globose, varying in size in the same culture from 3 by 4μ to 5 by 7μ. Neither sclerotia nor perithecia were found. Colonies grew well in all common media, grew better at 37°C. than at 20°C., liquefied plain gelatine (gelatine in distilled water) with yellow color in the liquid.

Culture: Cultures of a cottony, floccose type have been obtained from widely separated sources (no. 130 from Dr. B. F. Lutman, Burlington, Vermont; no. Sc. 171 in corn meal from Indiana; no. 2750 isolated by Johnston from mealy bugs in Porto Rico). Superficially these cultures show little relation to *A. flavus*. Microscopic examination of heads and spores, however, shows close relationships.

S. lutea Bainier (Bainier, Georges. Sterigmatocystis et Nematogonum. In Bul. Soc. Bot. France 27 (s. 2, t. 2): 27. 1880). See also Sartory, A., et Jourde, A. Compt. Rend. Acad. Sci. [Paris] 146: 548–549. 1908. Probably syn. of *S. lutea* Van Tieghem, but no acknowledgment in description; the same conclusion is expressed by Guéguen, Bul. Soc. Myc. France 15: 171–187. 1899.

Colonies at first beautiful yellow becoming greenish in age; mycelium forming deeply floccose masses up to 3 to 4 cm. in height and bearing conidiophores from the surface, both primary and secondary sterigmata were present, but measurements were not given except that the conidia were smooth, 6.4μ diam. Sartory and Jourde claiming the identity of their culture with *S. lutea* find the colonies yellow to green; stalks 300 to 800μ long; vesicles globose 12 to 30μ in diameter; and sterigmata in two series on certain media, primary 10 by 5 to 6μ, secondary 9 by 4μ, but said to be in one series in ordinary laboratory media; conidia yellow to green, globose smooth 3.5μ. This culture killed a rabbit when 2 cc. were inoculated through an ear vein. The smallness of the conidia in contrast to Bainier's description is not explained.

Culture: Bainier; Sartory and Jourde; no. 4640.473 received from the Bainier Collection in Paris is a strain of *A. flavus*, with conidia about 5μ in diameter, to which group the original probably belonged whether correctly represented in this culture or not.

SECTION XIII

Species Probably Aspergilli but without Sufficient Data for Grouping

For convenience these species with what we know about them are arranged alphabetically.

A. ageni. This name is cited by Lindt, Arch. Exp. Path. u. Pharm. 25: 265. 1889, as taken from Saccardo's Sylloge. Search for this reference leads to the conclusion that in this citation *A. hageni* was made to read *A. ageni.*

S. albo-lutea Bainier, Bull. Soc. Bot. France 27: 31. 1880. This was a small pale yellow form, but no further data were given and it has not since been identified. It might have belonged to the *A. flavipes* group, but no positive information is discoverable.

A. atrovirens Karsten, Symb. 26, p. 28; in Saccardo Sylloge 10: 524.

Saccardo gives: Stalks hyaline; heads cylindrical, cylindrical clavate or globose, dark green; "basidiis nullis suffultis, aeruginosis;" conidia globose, smooth, 4 to 6μ, green or greenish under the microscope; found on fruits of *Amygdalis communis* associated with *Eurotium lateritium.* While this may possibly indicate some member of the *A. glaucus* group, the description is not sufficient to justify maintaining the name.

S. chlorina Cooke and Massee, Grevillea 18: 7. 1889. Colonies on fruit of Citrus, effused, atro-fuscous; stalks erect (no measurements given); "with cuneate processes of the vesicle bearing 3 to 4 ellipsoid olivaceous basidia;" conidia globose, smooth, 5 to 6μ in diameter. Collected in E. New Guinea.

Culture: None.

We have found no report of further collections of *S. chlorina.* It is arbitrarily placed at 101 in the Key from description.

A. cookei (Cooke) Sacc. (Saccardo, P. A., Sylloge 4: 71. 1886.) = *A. mucoroideus* Cooke (Cooke, M. C. Australian fungi. In Grevillea, v. 12, no. 61, p. 9, 1883). Change of Cooke's name for bibliographic reasons.

Culture: None. To be discarded.

S. dubia (B. and Br.) Sacc., Fungi italici, pl. 902, and Sylloge 4: 72. = *A. dubius* Corda, in Berkeley and Broome, Notices of British Fungi,

Ann. Nat. Hist. 2 Ser., 7: 100, no. 520. 1851. This differs from Corda's organism in having both primary and secondary sterigmata, stalks figured as smooth, and in bearing conidia hyaline, globose, 4 to 5.5μ in diameter. Although described as white, Saccardo's table indicates a greenish color in the colony.

Culture: None. This organism has not thus far been recognized with certainty.

A. echinosporus Sorok. (Sorokin, N. W.[1] O njekotorych boljesnjach winograda i drugich rastenij Kawkasskago kraja. S. 22 tablizami rissunkow. Ottschjot, predstawlennyi w Ministerstwa Gossudarstwennych Imuschtschestw i Narodnago Prosswjeschtschenija. Tiflis 1892. Abstract by N. Busch in Zeitschrift für Pflanzenkrankheiten 3: 155. 1893).

Busch and Saccardo give: Entire fungus black; stalks continuous; sterigmata papilliform, very short; conidia in long chains, globose, coarsely muriculate, with connective, with the entire heads enveloped in slime.

Whether this species was a black Aspergillus or a species of another genus can not be decided from the information given, but the description of black heads enveloped in slime suggests the probability that the organism was not closely related to the usual forms of black Aspergli.

A. ferrugineus Fuckel, Fungi rhenani no. 157; also Symbolae Mycologicae in Jahrb. d. Nassauischen Vereins für Naturkunde Jahrg. XXIII and XXIV, p. 358. 1869–70. The description lacks specific information for identification. Colonies are designated "ferrugineis," stalks "longitudinaliter striatis," heads globose and conidia ovate small.

Culture: None.

This organism has not since been identified.

A fimeti Sacc., Fungi italici, pl. 703; Michelia 2: 543. 1888.

Colonies lutescent; stalks 800 to 1,000 by 20 to 25μ; sterigmata none or very delicate (?); conidia ovoid 8 to 10 by 6 to 7μ, hyaline, yellowish in mass. In hog dung, 1877.

Culture: None.

Although the size of the conidia suggests *A. glaucus*, examining Saccardo's figure gives no definite data to determine the exact group involved. The stalks figured are coarse and the contents of the upper half of stalk and head are yellowish. The proportions and colors suggest the *A. flavus* group. The name may be dropped unless more information as to its correct placing should be obtained.

[1] Not seen.

A. flavidus Berkeley and Broome, Fungi of Ceylon, Jour. Linnean Soc. [London], Bot. 14: 101. 1875. Cited by Saccardo as Fungi of Ceylon, London, 1871–73, no. 913s. Probably a member of the *A. flavus* group but not identifiable any more closely. Colonies "flavidus;" stalks, granular, unseptate; conidia elliptical 6 to 7μ in long axis.

Culture: None. To be dropped.

Badhamia fulvescens Cooke, Grevillea 4: 69, pl. 64, fig. 9. Dec. 1875. Cited as *Eurotium fulvescens* Cooke, Grevillea 8: 11. 1879–80; also Sacc. Syll. 1: 28. Perithecia tawny-ochre; ascospores pale brown, minute, ovate, 7.5μ

Culture: None.

The figure shows asci with the general appearance of the *A. glaucus* group but does not give data enough to identify the species.

Eurotium fulvescens Cooke, Grevillea 8: 11. 1880. Syn. *Badhamia fulvescens* Cooke. Change of generic designation only from the same material.

Not identifiable from descriptions.

A. fuscus Bonorden, H. F., Beiträge zur Mykologie. In Bot. Ztg., Jahrg. 19, No. 29, p. 202. 1861. Described from leaves of *Cornus alba* in Westphalia. Mycelium creeping on surface of leaves; stalks only emerging, erect, short, alternate at base, scarcely septate; heads ovate fuscous; chains radiating; sterigmata pearshaped. Conidia globose, fuscous, rough.

Culture: None; not again reported. The description suggests Haplographium but is certainly not definitely identifiable.

A. griseus Link, Sp. Plant., Ed. 4, Vol. 6, pt. 1, p. 69. 1824. Incorrectly cited by Bonorden and Wehmer as Link Obs. 1: 69. 1809. "Hyphasmate laniformi, sporidiis globosis. Habitat in variis corporibus putridis per Europam. Diagn. Lanam refert griseam parum lutescentem 2–3. lin. altam, e qua emergunt flocci sporidiferi frequentes hyphasma parum superantes. Capitulum parvum. Sporidia minuta copiosa colore a hyphasmate non diversa." The designations "laniform," "gray," might justify the suggestion that Link had some strain of the group containing *S. usta* Bainier.

Culture: Not cultivated. Possibly *A. ustus* or *A. versicolor*.

A. heterocephalus Spring, Bull. Acad. Roy. Belg., Sci. 19: 568. 1852. This name was given to colonies in a hen's egg which showed small heads globose and large heads columnar. Since no adequate figure or description was offered it may be discarded as a nomen nodum.

A. hispidulus Sprengel, Sys. Veg. Ed. 16, Vol. 4, p. 541, 1827. There is no suggestion of an Aspergillus in the description given.

A. incrassatus Spring, Bull. Acad. Roy. Belg., Sci. 19: 559, fig. 2. 1852. No characters to identify this form were offered.

S. lutea Van Tieghem, Bul. Soc. Bot. France 24: 103, 1877. All the information given is: A species with heads chamois yellow, different from *S. sulphurea* Fresenius, found upon date seeds left to germinate upon moss.

To be dropped.

A. micheli Preuss, Linnaea 25: 76. 1852. The description gives simple conidiophores, globose heads, etc., but no characters to separate the form intended from many other Aspergilli.

Culture: None. To be dropped.

S. minor Bainier, Bul. Soc. Bot. France 27: 30. 1880. Not separable even to group. All the data given are: Colonies green, total height of stalks and heads 252μ; sterigmata, primary 6.3μ, secondary 8.4μ long; conidia beautiful green, 2.4μ diameter.

Culture: Not reported; to be dropped.

A. mollis Berkeley, English Flora 5, pt. 2: 340. 1836. "(White branched Aspergillus); fertile flocci white, erect, dichotomously branched, apices clavate, sporidia large subglobose. On dead leaves. Winter. Apethorpe, Norths. Rev. M. J. Berkeley.—Forming minute, scattered, pure white fascicles, with thin procumbent mycelium." The description will not identify this as an Aspergillus.

A. mucoroides Corda, Icones Fungorum II: 18, fig. 76. 1837. No one since has reported this form. Corda's figure and description might designate a form either in the *A. glaucus* group or in the *A. flavus* group. A specimen labeled *A. mucoroideus* Cooke (q.v.) in Winter's Collection and belonging to the *A. glaucus* group might suggest a confusion between Corda's usage and Cooke's form, but furnishes no good ground for attempting to revive this specific name.

Culture: None; to be discarded.

A. mucoroideus Cooke (Cooke, M. C. Australian fungi. In Grevillea, 12: 9, no. 61. 1883). For bibliographic reasons became *A. cookei* Sacc. in Sylloge 4: 71. 1886. Cooke's diagnosis suggests some strain of the *A. niger* group; his note brings to mind certain strains of Syncephalastrum. A specimen from the herbarium of G. Winter in the Ellis Collection at the N. Y. Botanical Garden shows a member of the *A. glaucus* group close to *Eurotium chevalieri* Mangin with ascospores 4.8 to 5 by 4 to 3.5μ with deep furrow and crests. Conidia of *A. niger* were, however, also present in the preparations. It is probably impossible to decide what form Cooke had in hand.

Culture: None; to be discarded.

A. mülleri Berkeley, Jour. Linn. Soc. [London], Bot. XIII: 175. 1873. Described from Australian Lepiotas and other agarics: white (niveus); stalks subflexuous; conidia unequal, elliptical or obovate, very minutely roughened.

Culture: None, not elsewhere reported; to be dropped.

E. obliteratum Schw., Syn. Fungorum in Amer. Bor. n. 2725. This name was applied without adequate description to a perithecial form on badly dried herbarium specimens which was probably some member of the *A. glaucus* group. The name may be dropped for lack of information.

A. oblongisporus E. and E., no. 760, in Nuttall's Flora of Fayette County, West Virginia. Listed apparently by Millspaugh, in "The living flora of West Virginia," in W. Va. Geol. Survey 5 (A): 32. 1913 as *A. glaucus* var. *oblongisporus* E. and W. Examination of this material shows it to be a mixture of *A. flavus* and *A. repens;* the latter probably furnished the oblong spores responsible for the name.

Culture: None; to be dropped.

S. olivacea Van Tieghem, Bull. Soc. Bot. France 24: 103. 1877. Not identified with *A. olivaceus* Preuss 1852. The data given are "common," "heads olive-green" and "on cochineal;" it must be dropped for lack of information.

Culture: Inadequate; to be dropped.

A. olivaceus Preuss, Linnaea 25: 77. 1852. The data given are: Mycelium effused; stalk unbranched, straight, pellucid (= pitted?); heads small with short crowded elements, olive in color; conidia globose, minute, smooth, in olive-fuscous chains; upon moist Taraxacum powder. No basis for determination is found.

Culture: None; to be dropped.

A. periconioides Sacc., Ann. Mycol. 11: 320. 1913; no. 195 in Sydow, Fungi exotici exsiccati collected by P. W. Graff on leaves of Carica in Luzon, 1912.

As described: Stalks hyaline, erect, straight or flexuous, continuous, fuligineus 100 to 140 by 7 to 8μ; vesicles ovoid, pale fuligineus, about 14μ long; sterigmata in one series, radiate 8 to 9 by 2.5 to 3μ; conidia globose, smooth, "olivaceo-fuscellis," 3μ.

Culture: None.

Careful examination of this material showed several fungi to be present; some stalks and heads of the *A. glaucus* group were found, and occasional flexuous stalks probably representing the species described. Unless again collected and adequately studied the species may be disregarded.

S. prasina Bainier, Bull. Soc. Bot. France 27: 31. 1880. The information given is, *the color of the spores is pale green, their diameter 2.6μ;* to be discarded as a nomen nudum.

Cultures not known.

S. purpurea Van Tieg. (Van Tieghem, P. E. L., Sur le développement de quelques Ascomycetes. In Bul. Soc. Bot. France 24: 101–103. 1877.) This was not identified by Wehmer, Monogr. pp. 103–111. Van Tieghem noted, "very short stalks described as reddish brown or violaceous, spores of the same color and perithecia on bread." For these perithecia he describes a sclerotium phase followed by ripening of discoid ascospores. Could he have based the name upon finding purple ascospores among the conidia as we commonly do in mounts of *A. nidulans*?

Culture: On bread Van Tieghem. Compare *A. nidulans;* probably to be dropped.

A. quininae Heim. (Heim, F., Bul. Soc. Myc. France 9: 239–245. 1893.) Mycelium in quinine solutions dichotomously branched, stalk 3.5μ in diameter; vesicle 6 to 7μ in diameter, globose; conidia globose 3.5μ smooth.

Culture: Not studied except in quinine solutions.

Wehmer (Monogr. p. 91) is probably correct in deciding that no one can determine Heim's organism from this description. Whether a similar study of spoiling quinine solutions would bring certainty is problematical.

A. sphaerospermus Corda, nomen nudum. Icones Fungorum II: 18. Cited by Wehmer Monogr. p. 25, but no further data or references found.

A. spirius, cited by Amons, W. J. Th. Archief voor de Suikerindustrie in Nederlandsch.-Indië Jaarg. 29, Deel 1, p. 14. 1921.

Amons gives: Flesh colored; stalks 500μ long; conidia 3 to 4μ; without more information this name may be disregarded.

S. varia Bainier, Bul. Soc. Bot. France 27: 30. 1880. Described from carton and leaves of dried gilliflower.

Stalks and heads yellow; conidia green (!), surface growth 420μ deep; stalk 6.3μ in diameter; head 18.9μ; sterigmata, primary 4.2μ, secondary 8.4μ long; conidia 2.6μ.

Culture: Not reported.

Consideration of the description together with the failure of Bainier to recognize *A. nidulans* in any of his writings suggests that this species may have been a non-ascosporic variety of *A. nidulans.* It has never been recognized since the original description.

A. viridis, apparently undescribed, appears only on a specimen in the Curtis Collection now in the Cryptogamic Herbarium of Harvard University; the label is "*A. viridis* Schw., in herb., *A. virens* Lk. in Syn.: U. S., Herb. Schw." No Aspergillus could be found on the specimen.

To be dropped.

SECTION XIV

Species of Other Genera Described as Aspergilli; Other Species Probably of Other Genera but not Identified

A. africanus Dur. and Mont. = Nematogonum
A. alternatus Berk. = Stachybotrys
A. aurantiacus Berk. = Nematogonum
A. aureus = Nematogonium
A. bellomontii Mont. = Sporodinia
A. cimmerius Berk. and Curtis = Periconia
A. conoideus Sprengel = a myxomycete
A. crocatus B. & C. = Chondromyces
A. cucurbitaceus (Curtis Collection) = Choanephora
A. curtisii Berk. = Rhinotrichum
A. dasytricha E. & E. = unidentified
A. echinosporus Sorokin = ?
S. ferruginea Cooke = undetermined
E. insigne Winter = ? Gliocladium
A. laneus Link = undetermined
A. laneus in Schweinitz = Rhinotrichum
A. maximus Link = Sporodinia
A. penicillatus Grev. = *Briarea elegans* Sturm (Sacc. Syll. 4: 85); see *A. glaucus* group
A. pouchetii Mont. = Sporodinia
A. pulvinatus B. & C. = undetermined
A. purpureofuscus Fries = undetermined
A. quadrifidus Link = Botrytis
A. racemosa Persoon = ?
A. roseus Batsch = undetermined
A. simplex Persoon = Penicillium ?
S. skottsbergii Bresadola and Vestergren = ?
E. stercoraria = *Anixiopsis* Hansen

A. africanus Dur. and M., Fl. Alg. I: 342. 1849. Not probably an Aspergillus. Described from an Opuntia from Algieria; material had been dried and its condition was not satisfactory for the preparation of figures. From the description, this species has been variously referred to Nematogonum, Botrytis, and Acmosporium.

Culture: None.

A. alternatus Berkeley, Ann. Nat. Hist., ser. 1, 1: 262. 1838. Berkeley's figure excludes Aspergillus, and apparently agrees with *Stachybotrys alternatus* Bonorden.

Culture: None.

A. aurantiacus Berk., British Fungi, fasc. IV. 1843. Cited by Montagne, Ann. Sci. Nat. Bot., ser. 3, 12: 299. 1849. Syn. *Nematogonum aurantiacum* Desm. See Farlow's Bibliographical Index, vol. 1, pt. 1, p. 276. 1905. The figures and description exclude this species from Aspergillus. Specimen No. 2428 under this name in the Curtis Collection now in the Cryptogamic Herbarium of Harvard University is identified by Professor Thaxter as a Rhinotrichum.

A. aureus Berkeley (Berkeley, M. J.). English Flora 5: 346. 1836. This species is cited in Saccardo Sylloge 15: 53. 1901, as Nematogonium[1] aureum Berk.

A. (Sporodinia) bellomontii Montagne, Ann. Sci. Nat. Bot., ser. 4, t. 12: 181–182. 1859. = Sporodinia sp. Noted as related to *A. maximus*. Not an Aspergillus.

A. cimmerius Berk. and Curtis, Grevillea 3: 108. 1875. Dead cabbage leaves, 1859. Dundee, S. H. Wright in the Curtis Collection. Type specimen, No. 41. This specimen was identified by Dr. Farlow, Bibliographical Index I, pt. 1, p. 277, 1905, as *Periconia chlorocephala* Fres. We have seen Dr. Farlow's preparation from this material.

A. conoideus Sprengel, Sys. Veg., ed. 16, 4: 541. 1827. This is cited as *Byssus conoidea* Müll. Flora danica, table 897, fig. 2, which is a myxomycete, hence the name may be dropped.

A. crocatus B. and C. Specimen only in the Curtis Collection. This type specimen has been identified as Chondromyces and is so reported in Farlow's Bibliographical Index I, pt. 1, p. 277. 1905.

"*A. cucurbitaceus*, ad juniores cucurbitas verrucoses," etc., autumn, 1870 in the Curtis Collection. = Syn. *Choanephora cucurbitarum* Berkeley and Ravenel, Grevillea 3: 11. 1875. See discussion and synonymy by Thaxter, R., Rhodora 5: 98–102. 1903.

A. curtisii Berk. On trunks of Myrica, no. 83, in Ravenel Fungi Caroliniani, fasc. IV. 1855. This is cited by Farlow in his Bibliographical Index vol. 1, pt. 1, p. 277, 1905, as *Rhinotrichum curtisii*. Two packets on this sheet and the one marked 206B from Houston, Texas, 1869, are the same type.

Culture: None; to be dropped.

S. dasytricha Ell. and Ev., Jour. Myc., p. 104. 1886; Sacc. Sylloge X, p. 525. "On decaying wood, lying on the ground. May. Langlois, No. 441."

[1] Authors using this name differ in its spelling. Nematogonium and Nematogonum are both found.

"Fertile hyphae, effused, velutinous, erect, pale, olive-brown, septate, 250 to 300 by 6 to 8μ, the oblong or ovate, enlarged apex thickly covered with coarse, nodulosely-branched sterigmata, 20 to 25 by 4μ, obtuse and sublobate at the tips, and with short, rudimentary, lateral branches or projections, which are often little more than mere swellings or tubercles, irregularly arranged and all together, forming an oblong head, 45 to 60μ long by 20 to 25μ thick; conidia oblong-cylindrical, hyaline, 5 to 7 by $1\frac{1}{4}$ to $1\frac{1}{2}\mu$, borne either singly or 2-3-catenuate on the tips of the basidia. Under the lens or even to the naked eye, the conidia are white, causing the olivaceous, velutinous hyphae to appear sprinkled with gray. The general appearance is that of *Menispora glauconigra* C. and E." Conidia up to 5 to 7 by up to 2.5μ.

Culture: None. Not an Aspergillus in the sense of this paper.

S. ferruginea Cooke, Jour. Quekett Micros. Club, 2 ser., 2: 139. 1885. Cooke himself admits that this is not an Aspergillus or Sterigmatocystic as he has described it. Proper classification would require another collection and culture of the organism. The specific name had already been used for an Aspergillus by Fries and by Fuckel.

Culture: None; to be dropped.

A. globosus Link = *Sporodinia aspergillus* Schröter, in Sacc. Syll. 7: 207.

Eurotium insigne Winter, exsiccati in Rabh. Fungi no. 1732, description on label and in Hedwigia 1873, also in Rabh. Krypt. Fl. Aufl. 1, Abt. 2, 2: 61. 1887. A perithecial form described from goose dung, not showing a conidial form, but believed by Winter to be connected with Gliocladium; ascospores described as globose, almost colorless, rough, 14 to 16μ in diameter.

Culture: None.

The specimen in the Pathological Collections of the United States Department of Agriculture was examined but no ascospores were found.

A. laneus Link, Obs. p. 16. 1809. Link gives: Section "b. Floccis ramosis; *A. laneus*, caespitibus densis, floccis implexis e luteo-albis erectiusculis, capitulis lutescentibus. In fungis putridis caespites magnos format, floccis ultra lin. longis valde ramosis. Capitula magna globosa. Invenit am. Ditmar." The description given by Link cites no previous author. The same description is repeated by Link in Spec. Plant., ed. IV, 6: 66. 1824. Sprengel (Sys. Veg., ed. 16, 4: 541. 1827) gives *A. laneus* Link as a synonym for *Syzygites megalo-*

carpus Ehrenberg. Saccardo, Sylloge 4: 136 gives it as a synonym for *Botrytis lanea* (Bon.) Sacc. This name is used on no. 1277 in De Thümen Mycotheca Universalis, but the organism is not an Aspergillus. *A. laneus* in Schweinitz, Syn. Am. Bor. is identified by Berkeley in Grevillea 3: 108, 1875, and by Farlow, Bibliographical Index, vol. I, pt. I, p. 279. 1905, as *Rhinotrichum curtisii* Berkeley. Admitting the possibility that *A. effusus* Tiraboschi might represent this form, it does not seem desirable to accept the possibility as warranting the identification. Wehmer (Monograph) suggests *A. glaucus*. There seems to be little chance of determining what organism was described under this name by Link, although Saccardo (Syll. 4: 136) gives the name as a synonym for *Botrytis lanea* Bonorden.

A. maximus Link, Obs. p. 16. 1809. "*A. maximus*, caespitibus effusis, floccis dichotomis badiis, sporidiis maximis. Caespites magnos format in fungis putrescentibus, crassos. Flocci majores ac in hoc ordine solent, ramis divaricatis. Sporidia crebra, microscopio simplici detegenda. Invenit am. Ditmar Iconem v. fig. 15." This name is cited by Fries, Sys. Myc. 3: 38. 1829; by Berkeley in British Flora (Hooker), vol. 5, pt. 2, p. 340. 1836, who identifies with it *A. laneus* of Greville Fl. Ed. p. 467. There seems to be little doubt that the name was used for a Sporodinia, not an Aspergillus. This usage occurs (see Farlow, Bibliographical Index, 1, pt. 1, p. 279. 1905) on a specimen collected by Ravenel in South Carolina and preserved in the Curtis Collection at Harvard.

Name to be dropped.

A. pouchetii Montagne, Ann. Sci. Nat. Bot., 4 ser., t. 12: 182–183. 1859. As described, this was one of the Mucorini noted as having resemblances to *A. maximus*.

A. pulvinatus B. and C. Original collection as far as seen appears to be in the Curtis Collection from Society Hill, S. C., 1855, also one marked F. cub.-Wright no. 642 in the Curtis Collection; another series of specimens marked B. and C., no. 1648 in Ellis and Everhart, N. A. Fungi, collected on dead twigs at Newfield, N. J., 1885; and probably the same as no. 2306 in the Ellis Collection. This fungus as represented in collections, forms brown pulvinate masses conspicuously fringing dead twigs for several centimeters in length and 2–3 mm. in height, consisting of hyphae 7 to 8μ in diameter with walls brown, rough, septate, dichotomously branching and producing terminal and intercalary fruiting areas about 100μ long upon which primary and secondary series of vesiculose cells terminate in long conidia-bearing

sterigmatic tubes from which are cut off conidia 7 to 8 by 4 to 5μ, with brown, rough, fairly thin walls.

Correct placing of this species can only be based upon fresh material, but it is certainly not an Aspergillus in the sense of this paper. For the purpose of tracing existing collections the organism is arbitrarily grouped, however, in the Key.

A. purpureofuscus Fries (Fries, E. M. Systema Mycologicum, v. 3, p. 388. Gryphiswaldae, 1829). Evidently described from Schweinitz's material. (Schweinitz, L. D. von. Synopsis fungorum in America boreali media degentium. Secundum observationes. In Trans. Amer. Phil. Soc., n. s., v. 4, p. 282, no. 2680. 1834.) Schweinitz's description follows: "*A. purpureofuscus*, L. v. S., species distinctissima. In caulibus putridis Brassicae rarius occurrit, Bethl. A. Hyphasmate longe lateque effuso, tenue. Floccis sporidiferis ramosis et erectis, aut etiam simplicibus, septatis purpureo-fuscis. Sporidiis creberrimis, concoloribus, demum canescentibus, majusculis. Elegans ac persistens." Type specimen marked in Curtis Herbarium "Schw." Appears as a crusted, evidently somewhat gelatinous growth on the side of a semi-rotten stem of some species of Brassica; in slides shows separate coremia of 5 to 50 hyphae about 2 microns in diameter with purple walls, septate, packed together in parallel, scarcely twisted aggregations and reaching a length of about 300 microns; with fruit heads terminal on branches; length of stalks not determined; conidia 3.3 to 4.5 by 6 to 7μ; brownish, heavy walled, elliptical. Exact nature of heads not successfully worked out, but not that of a true Aspergillus. Exsiccati: Schweinitz material only.

Culture: None. Identification doubtful, suggests Myriocephalum (DeNot.) Mont.

Aspergillus quadrifidus Link. Obs. 2: 36. 1816.

This species was described as having the spore-producing hyphae (stalks) quadrifid at the apex; Saccardo gives this as a synonym for *Botrytis ramosa* Pers. in the Sylloge 4: 133. 1886.

Aspergillus racemosa Persoon, Neues Mag. Bot. 1: 121. 1794. Tentamen Dispositionis Methodica Fungorum, etc., p. 41. 1797. This is excluded by description "nigrescens, stipite racemosa;" it is not recognizable.

A. roseus Batsch, Elenchus fungorum, no. 58, p. 183, fig. 58; cited by Link, Spec. Pl., ed. IV, t. VI, pt. 1, p. 68. 1824; also by various authors for a rosy or flesh colored organism, and by Corda as *Haplotrichum roseum* in Pracht-flora, pl. XI. From the figures and descriptions this was not an Aspergillus in the sense of this paper.

A. simplex Persoon, Tent. Disp. Meth. Fung., etc., p. 41, 1797. The information given, "glaucus, stipite simplice," would not identify an organism, although tradition considers this a Penicillium

S. skottsbergii Bresadola and Vestergren, no. 250 in Vestergren, Micromycetes rariores selecti Rossia baltica: ins. Osilia, Kielkond in silva abiegna prope Kattiel in follis vivis *Aquilegiae vulgaris*, 1899; distributed without description; examined in collaboration with Professor Thaxter at the Harvard University Cryptogamic Herbarium did not prove to be an Aspergillus in the sense of this paper, but was not identified.

E. stercoraria E. Chr. Hansen. Meddelelser fra den Naturhist. Foren. i Kjöbenhavn, p. 310, 1876. Syn. *Anixiopsis stercoraria* E. Chr. Hansen, Bot. Ztg. 55: 127–131, Taf. II, fig. 8. 1897. Collected upon fox dung in 1874, transferred and cultivated on laboratory media in 1895. No Aspergillus-like conidial apparatus appeared in the course of extensive cultural study. The asci and especially the shape and markings of the ascospores have no resemblance to the known species of Aspergillus.

SECTION XV

The Synoptical Key to the Aspergilli

In the key which follows, the great groups recognized are based upon actual culture of Aspergilli. The literature has been interpreted (see Chapter III) and species described fully enough but not recognized in culture are allotted to their proper places in these known groups. Other species less completely described but recognizable by their group relationship are placed within the group more or less arbitrarily in harmony with the data given in the description. Some descriptions apparently accurate but not readily harmonized with membership in groups known in culture have been given arbitrary places in the key to accord with the data. Some forms not at first known in culture but for which places were created to fit the descriptions, have since been found. Others undoubtedly will be obtained in the future. Necessarily there are many places in the proposed system at which our information has been thus far inadequate and has led to the allocation of species to wrong sections of the group, or to the suggestion of two or even three possible places for certain organisms not known to us in culture. Finally, some descriptions are so inadequate that even a guess at group relations seems hardly justified, although the guess is occasionally offered.

NATURAL SEQUENCES MAINTAINED

Real relationship is indicated as completely as possible not only in the order of presentation of groups but wherever possible within the groups themselves. Some of the separating characters are necessarily arbitrary, but wherever possible, organisms believed to be closely related have been placed in proper sequence and diagnostic contrasts devised to avoid separation of these closely allied forms.

ABBREVIATIONS

Latin names, symbols, arbitrary names or record book numbers used to designate species described or strains of Aspergilli studied appear in italics. The prefix A. stands for Aspergillus; S. indicates a

form described as Sterigmatocystis; E. a species of Eurotium. Abbreviations of authors' names follow the usages encountered in other taxonomic papers.

INDEX NUMBERS

In the dichotomous number system adopted, the index number in the column at the left is duplicated wherever two contrasting characters are made a basis for separating groups. Numerical order is rigidly maintained in the column at the left. The numbers at the right indicate the position of the continuation of each idea in the column arranged numerically at the left. A descriptive line is occasionally repeated under a single number where continuity has been broken or where such repetition appears to clarify the characterization of a group.

SYNOPTICAL KEY

1.	Heads definitely cylindrical clavate, fertile area (vesicle) cylindrical, much longer than the diameter, not globose or elliptical.....	2.
1.	Heads not clavate.....	9.
2.	Stalk walls colorless.....	3.
2.	Stalk walls brown—dematiaceous.....	8.
3.	Sterigmata in 1 series.....	4.
3.	Sterigmata in 2 series.....	6.
4.	Stalks coarse, fairly long, usually several mm. on all media; represented by no. *107* and many other isolations.....	*A. clavatus*, p. 97.
4a.	Apparently synonymous.....	*A. clavellus*, p. 99.
4b.	Name attached to specimen apparently near *A. clavatus*.....	*A. westendorpii*, p. 99.
4c.	Stalks very short (dwarf) on Czapek, 1–2 cm. on gelatine and dung cultures.....	*A. giganteus*, p. 99, 138, *4754.3.*
4d.	Stalks very short, slender; persistently dwarfed form.....	? *4202.6c.*
4e.	Conidia 2 by 5 to 6μ.....	*A. fusco-cinereus*, p. 100.
6.	Sterigmata in 2 series.....	7.
7.	Ascosporic, general morphology suggesting *A. clavatus*.....	*A. pseudoclavatus*, p. 100.
8.	Stalks smooth, brown, septate, thick-walled, with terminal and some intercalary conidial masses; sterigmata 1 series, conidia colorless 2 by 5 to 7μ.....	*A. dasytricha*, p. 216.
9.	*Heads not clavate*, 10.	

10. Stalks dichotomously branched, brown, rough, septate; conidial masses both terminal and intercalary; sterigmata 2 series, conidia subglobose 6 to 8μ..... *A. pulvinatus*, p. 218.
10. Stalks not dichotomously branched..... 11.
11. Stalks fused at base into coremiform masses, spreading out to bear separate heads..... 12.
11. Stalks not fused into coremiform masses..... 15
12a. Colonies bright egg-yellow..... *S. vitellina*, p. 187.
12b. Colonies purpureofuscus..... *A. purpureofuscus*, p. 219.
15. Stalk walls smooth, not pitted or roughened (conidia either rough or smooth)..... 16
15. Stalk walls pitted, sometimes also granular; commonly reported as rough, asperulate, or spinulose; pitting nearly obliterated in some forms..... 220

Note—(Superficial granules or crystalline concretions are often found, but no pits or roughenings of the stalk wall occur in *A. glaucus* series.)

16. Conidial heads some shade of green when young. (Exceptional but obviously or probably related forms included (See 51)..... 17
16. Conidial heads never green..... 140
17. Sterigmata in 1 series..... 18
17. Sterigmata in 2 series..... 100
18. Conidia predominantly more or less elliptical or pyriform (occasionally globose?) mostly more than 4μ in long axis. Exception *A. penicilloides* (See 61) and *A. amstelodami* about 4μ and globose..... 19 to 53 *A. glaucus* group, p. 101.
18. Conidia mostly globose; usually less than 4μ in long axis..... 80
19. Conidia mostly pitted or rough (exception see nos. 27c and 27d)..... 20
19. Conidia described as smooth..... 65
20. Perithecia yellow to orange, produced in sucrose media, superficial, brittle; De Bary's Eurotium (*A. glaucus* series in part). Asci and ascospores develop fairly rapidly in cultures of most species under the usual conditions of the laboratory..... 21
20. Perithecial forms not adequately described... 41
20. Perithecia not reported..... 42
21. Ascospores ranging in long axis from 11 to 8.4μ. 22
21. Ascospores ranging in long axis from 8.4 to 6.1μ..... 26

21. Ascospores 6μ or less in long axis.....	30
22. Ascospores with furrow and crests or frills....	23
22. Ascospores with furrow but without frills....	25
23. Ascospores 9 to 10 by 6 to 7.5μ with furrow and crests.....	*A. herbariorum* ser. *major*, p. 106.
23a. Ascospores about 11 by 7μ with furrow and markings on valves.....	*A. echinulatus*, p. 107. = *A. brunneus*, p. 107.
23b. Ascospores 7 to 10μ with frill; perithecia brick-red.....	*E. lateritium*, p. 107.
23c. Ascospores with markings or frills on surface of valves.....	*E. verruculosum*, p. 107.
23d. Culture from figs in California; verified by Vuillemin as *E. verruculosum*.....	*4481*
23e. Ascospores 9.4 by 6.6μ; conidia 15 to 18μ.....	*A. herbariorum* ser. *major* var. *violaceus*, p. 106.
23f. Ascospores up to 10 by 6μ; conidia up to 12 by 4 to 6μ.....	*A. medius*, p. 108.
23g. Ascospores 11.2 by 5 to 6μ, with valves smooth and conidia smooth.....	*4667.86.*
23h. Perithecia 225μ diameter; conidia rough, 14 to 17μ; pigment red; ascospores 11.2 by 5 to 6μ with valves smooth.....	*A. disjunctus*, p. 108.
23i. Sartory notes that *A. godfrini* Sartory and Roederer resembles this species in morphology, hence perhaps belongs here; compare 267c.....	*A. godfrini*, p. 109.
25. Ascospores with furrow definite but without crest or frill.	
25a. Ascospores 11.2 by 6μ; perithecia 140μ diameter; conidia very variable; pigment red....	*A. repandus*, p. 109. = *4167.*
25b. Ascospores 10 by 4.7μ, markings not mentioned; perithecia 200 to 220μ diameter; conidia 9 to 12μ, markings not mentioned; pathogenic...	*A. mencieri*, p. 109.
26. Ascospores ranging from 8.4 by 6.1 to 6.6 by 5.6μ, with furrow and crests or frills.....	*A. herbariorum* series *minor*, p. 110.
26. Ascospores ranging from 8.4 by 6.1 to 6.6 by 5.6μ, without crests.....	27.
27a. Colonies dark green in growing period; perithecia 168μ diameter; conidia 5 to 9μ globose; pigment red.....	*A. umbrosus*, p. 110.
27b. Fairly represented by culture from Miss McCormick.....	*4803.1914.*
27c. Colonies at first canary yellow, then through ochraceous to yellow-green, green and dark	

green; perithecia 200μ, not abundant; ascospores with furrow; conidia smooth, very long, elliptical, very variable in size; pigment red (See also 53)..... *A. mutabilis*, p. 110.

27d. Perithecia 140 to 168μ diameter, very numerous; conidia long elliptical, 14 by 8.5μ; pigment red (See also 53)..... *A. mollis*, p. 111.

27e. Culture series falling in this group.

1. Ascospores 6.8 to 6 by 6.4 to 5.2μ..... *Culture 134.*
2. Ascospores 6 to 6.9 by 5.6μ..... *4168.*
3. Ascospores 5.2 to 6.9 by 3.9 to 4.3μ..... *4194.*
4. Ascospores 6 to 7.7 by 5μ..... *Cornstalk.*
5. Ascospores 6 by 4.3μ..... *Cork MMO*

30. Ascospores 6μ or less in long axis..... 31.

31. Ascospores with furrow and crest or frill (ridges)..... 32 a to h.

31. Ascospores without crest (ridge or frill)..... 33.

32a. Ascospores 4.4 to 5 by 3 to 4μ..... *4122.1.* *4706.Ac5.*

32b. Ascospores 3.6 to 4.3 by 3.9 to 4.3μ..... *4122.2.*

32c. Ascospores 6.2 to 5.2 by 3.8 to 4.2μ..... *4125.3.*

32d. Ascospores 6.2 to 5.2 by 3.4 to 4.3μ..... *4138L32.*

32e. Ascospores 6 to 5.1 by 4 to 4.3μ..... *4193.*

32f. Ascospores 4.7 by 3.7μ..... *A. chevalieri*, p. 111.

32g. Ascospores 4.7 by 3.7μ, apparently *A. chevalieri* *4684P.*

32h. Ascospores 4.8 to 5 by 3 to 3.5μ, specimen from herb. G. Winter in Ellis Collection labeled *A. mucoroideus*, p. 211.

33. Ascospores without crest (ridge or frill)..... 34.

34. Ascospores 5 to 6μ in long axis..... 35a to h.

34. Ascospores less than 5μ in long axis..... 36.

35a. Colonies deeply floccose, felted, slowly becoming clear green and finally reddish brown; perithecia abundant about 156μ diameter; ascospores 5.6 by 4.2μ; conidia 2.8 to 5.6μ; pigment red..... *A. sejunctus*, p. 111.

35b. Colonies slightly floccose in center only, becoming brown; perithecia few, in dry areas 120 to 175μ; ascospores with furrow, varying from 5 by 4μ or 5 to 6 by 4 to 5μ, culture from Raistrick..... *4724.39.*
This culture was labeled (with what evidence?) *A. ferrugineus* Fuckel p. 209.

35c. Colonies quickly green, then becoming yellow from perithecia in center and finally rusty red; conidia 5 to 8μ, rough; perithecia 80 to 100μ diameter; ascospores 6 by 4 to 5μ with furrow shallow..... *A. ruber*, p. 112.
Yellow pigment.

35d. Ascospores 5.6 by 4.5μ with furrow more or less evident; perithecia 126μ diameter; conidial areas green, stalks short, conidia 4.2 to 8.4μ; pigment yellow..... *A. scheelei*, p. 112.

35e. Ascospores 5 by 4μ without ridges, with furrow difficult to demonstrate; perithecia 98μ diameter; pigment yellow..... *Asp. B.* var. *scheelei*, p. 113.

35f. Ascospores 5 to 6 by 4 to 5μ; yellow pigment; perithecia small. From Raistrick, labeled *A. novus* Wehmer (?CT.)..... *4724.46.*

35g. Ascospores 5.2 by 3.5 to 4.2μ..... *4013.*

35h. Ascospores 5.2 by 3 to 4μ..... *4194.*

36. Ascospores less than 5μ in long axis..... 37.

37. Ascospores 4.7 by 3.7μ with distinct furrow only, no ridges..... 38a and b.

37. Ascospores 4.7 by 3.7μ without definite furrow or ridges..... 40.

38a. Conidia 2.5 to 4.5μ globose, echinulate..... *A. amstelodami*, p. 113.

38b. Fairly represented by the Amsterdam culture. *109.*

40. Ascospores 4.7 by 3.7μ with edge thick, no crests and *no* furrows ? CT.; conidia 4.7 by 7.6μ globose..... *A. repens*, p. 113.

Culture with this morphology, one of many seen..... *4695.9.*

41. Perithecial forms without full description.

41a. Ascospores described as globose, colorless, rough, 14 to 16μ diameter, not an *Aspergillus* !..... *E. insigne*, p. 217.

41b. Cited from Martius by Link..... *E. fructigenum*, p. 115.

41c. Colonies at first yellow then fuscous red; perithecia small, abundant, on wood in Germany and Italy..... *E. epixylon*, p. 115.

42. Perithecia not reported. Species inadequately described but placed here from fragmentary information only, not identifiable, hence to be discarded..... 43.

43. Conidia large in size and with markings as in *A. glaucus* group, colonies variously colored 44.

43. Conidia small 3 to 5μ..... 60.

44. Conidial masses enveloped in slime..... 54.

44. Conidial masses not enveloped in slime..... 45.

45. Species known only as pathogens..... 45a and b.

45a. Conidia 3 to 12μ..... *A. tokelau*, p. 115.

45b. Conidia 4 to 6 by 3 to 5μ..... *A. fontoynonti*, p. 115.

45. Species not known as pathogenic..... 46.

46. Species described as varieties of *A. glaucus* or placed here on account of size, and markings

	of conidia, general type of head and stalk disregarding color reports on account of the confusion frequently noted between color of conidial heads and the coloration of colonies, by encrustment of hyphae or by secreted pigment. Most of these forms would be difficult or impossible to identify.....	47.
47.	Recognized by describers as forms of *A. glaucus*	48a to f.
47.	Described without indicating any relationship to *A. glaucus* group.....	49.
48a.	Heads olivaceous green; conidia globose, rough, 8 to 10μ diameter.....	*A. glaucus* var.* *olivascens*, p. 119.
48b.	With color white and a little larger than *A. glaucus*.....	*A. glaucus* var. *albida*, p. 119.
48c.	Green to greenish brown; conidia 6 to 17 by 6 to 12μ, rough.....	*A. glaucus* var. *minimus*, p. 119.
48d.	"Subolivaceus;" conidia 7 to 9.5 by 6 to 8.5μ..	*A. glaucus* var. *subolivaceus*, p. 119.
48e.	Conidia oblong-elliptical, 5 to 7.5 by 2.5 to 3μ.....	*A. glaucus* var. *oblongisporus*, p. 212.
48f.	Later separated by De Bary as a species.....	*A. glaucus* var. *repens* p. 113.
49.	Species with conidial form fairly well described	50.
49.	Species placed here on account of fragmentary information pointing to the relationship as probable.....	53a to h.
50.	Colonies becoming dark reddish brown in various shades.....	51a to e.
50.	Colonies in shades of yellow to brownish.....	52a to d.
51a.	Conidia echinulate, globose, 15μ or ovoid 12 to 18 by 8 to 15μ..... (Possibly referable to *A. tamarii* group ? which see.)	*A. brunneofuscus*, p. 117.
51b.	Conidia globose, echinulate, 15μ diameter.....	*A. brunneus*, p. 107.
51c.	Ascogenous phase identified as *A. echinulatus*.	p. 107.
51d.	Conidia given in Michelia 15 to 18 by 12 to 43μ, in F. ital. fig. 17, as 12 to 13 by 15 to 18μ; see 281d.....	*A. ochraceo-ruber*, p. 120.
51e.	Conidia 10 to 12 by 10μ, stalks up to 1,500μ long, often dichotomous at apex.....	*A. rufescens*, p. 121.
52.	Colonies in shades of yellow to brownish.	
52a.	Conidia 8 to 12μ, globose or elliptical, colonies yellow to brownish yellow.....	*A. giganto-sulphureus*, p. 118.

52b. Conidia 8 to 10 by 6 to 7μ, yellowish in mass. *A. fimeti*, p. 209.
52c. Colonies shades of orange-yellow, isabelline to deep olive buff; conidia 9 to 10μ in long axis, figured as smooth; compare 281a ..... *A. sartoryi*, p. 122.
52d. Stalks yellow, conidia yellow, mostly 10 to 12μ, range 8 to 15μ in long axis ..... *A. spiralis*, p. 122.
53. Species very inadequately described but probably belonging here.
53a. Conidia large; stalk figured as septate and slightly constricted at the septa; on black bread—Westphalia. *A. macrosporus*, p. 120.
53b. Conidial form on badly dried grass in the herbarium; canescent colonies; figured by Greville ..... *A. penicillatus*, p. 121.
53c. Same reproduced by Link ..... *A. penicillatus*, p. 121.
53d. Canescent colonies; coarse stalks 600 to 800 by 20μ; conidia ovoid to globose, 7 to 10 by 6 to 7μ ..... *A. stercoreus*, p. 122.
53e. "Floccis ferrugineis nitidis;" smaller than *A. glaucus;* Link's species ..... *A. ferrugineus*, p. 118.
53f. Apparently a repetition of Link without additional information; Fries' species ..... *A. ferrugineus*, p. 118.
53g. Effused, white, floccose, felted, with oval spores ..... *A. ovalispermus*, p. 161.
Synonym, fide Farlow, Bib. Index ..... *A. oosporus*, p. 161.
53h. Upon the fat in cooked food.
Mycelium densely floccose; heads large; Link's species ..... *A. virens*, p. 123.
54. Conidial masses becoming enveloped in slime (See also 67f) ..... 55.
55. Colonies green, short stalked, with conidia 4 to 6 by 3 to 3.5μ, occasionally up to 8μ in long axis, smooth or echinulate ..... *A. conicus*, p. 125.
60. Conidia small, otherwise as in *A. glaucus* group. 61.
61. Species growing only in highly sugared media; conidia 3 to 4μ, mostly globose ..... *A. penicilloides*, p. 126.
61a. Culture from Louisiana, verified by Spegazzini ..... *4197.3*, and *N.0.7*.
Cultures near to this species from cigars ..... *4814*.
61. Species growing under other conditions; an unrelated or arbitrary series ..... 62.
62a. Species found in peanuts, conidia 4 to 5μ green to brown; stalks slender sinuous ..... *A. brunneo-virens*, p. 126.
62b. Conidia 3 to 4μ delicately roughened; stalk coarse ..... *A. varians*, p. 127.
65. Conidia smooth, otherwise *A. glaucus*-like .... 66.
See also numbers 27c and 27d.
66. Ascosporic ..... 68.

66. Non-ascosporic..... 67a to f.
67a. Conidia 2.8 to 5.9 by 11.2μ or 2.8 to 11.2μ in a calyptrate column..... *A. cinerescens*, p. 118.
67b. Conidia 6 to 8 by 3 to 4μ hyaline to rosy..... *A. carneolus*, p. 117.
67c. Conidia 4 by 7μ, stalk 100 to 200 by 4 to 6μ.. *A. caesiellus*, p. 117.
67d. Conidia 5 to 6.6 by 3.5 to 4μ..... *A. olivaceus* Delacr. p. 120.
Syn. *A. delacroxii*, p. 118.
67e. Conidia 6 to 10μ..... *A. argentinus*, p. 116.
67f. Conidia 4 to 4.5 by 2.5 to 3.5μ, smooth or occasionally roughish in age, forming dark slimy, deep green masses..... *A. conicus*, p. 125.
Possibly *A. cinerescens*, p. 118.
67h. Cultures belonging with *A. conicus*..... *2502*, *4733.70.1*, *4733.64.1*
68. Ascosporic forms..... 69.
69. Conidia elliptical or oval smooth..... 70.
69. Conidia globose..... 73.
70. Conidia long elliptical..... 71a and b.
70. Conidia oval..... 72.
71a. Perithecia about 200μ diameter, sparingly produced. See no. 27c..... *A. mutabilis*, p. 110.
71b. Perithecia about 140 to 168μ, abundant (also no. 27d)..... *A. mollis*, p. 111.
72. Conidia oval, 5μ in long axis; stalks greenish sinuous; perithecia 100 to 150μ diameter, subglobose; ascospores 4 to 6μ in number, smooth, sphaeroidal hyaline..... *A. belfanti*, p. 124.
73. Conidia globose.
73a. Conidia 3.5 to 4μ globose; perithecia 70 to 80μ white; ascospores 6 to 8μ..... *A. cinereus*, p. 126.
73b. Conidia 3.5 to 4μ (smooth? no record); perithecia yellow 100 to 125μ; ascospores 6 to 8μ..... *A. subgriseus*, p. 124.
73c. Possible synonym..... *E. subgriseum*, p. 124.
73d. Conidia globose, smooth, 4.3μ; ascospores smooth, round, 4μ (?) sense of Eichelbaum. *A. virens*, p. 123.
80. Conidia globose or subglobose, mostly small, rarely 4μ or over in long axis (compare no. 73); conidiophores mostly not over 500μ long and 3 to 6μ diameter. Exception 88a..... 81. Wehmer's *Microaspergillus in part.*
81 Species otherwise with characters of *A. glaucus* group. See nos. 61 and 62b.
81. Species not readily confused with *A. glaucus*. 82.
82. Heads radiate..... 83.
82. Heads forming columnar (calyptrate) masses, consisting of parallel adherent chains of conidia..... 85.

83a. Conidia oval 2μ. See also 143b.....	*A. minimus*, p. 127.
83b. Conidia oval 3μ. See also 143c.....	*A. koningi*, p. 127.
83c. Conidia globose 3 to 4μ, stalks fuligineus.....	*A. periconioides*, p. 212
85. Heads forming columnar (calyptrate) masses consisting of parallel adherent chains of conidia.....	86.
86. Conidia barrel-form to subglobose 3μ; blue-green, citromyces-like.....	*A. gracilis*, p. 125.
86a. Culture agreeing with this description (not now viable).....	*4246.*
86. Conidia globose.....	87. *A. fumigatus* group, p. 129. Nos. 86 to 92.
87. Not ascigerous.....	89.
87. Ascigerous.....	88a to f.
88a. Ascospores 6 to 8μ, lens shaped with groove or furrow; stalks up to 1,000μ, heads radiate; conidia 3 to 4μ.....	*A. malignus*, p. 132.
88b. Ascospore with central body, 4 by 5μ with band or frill extending 1 to 2μ further, hence 5 by 7μ with band.....	*A. fischeri*, p. 132.
88c. Culture from Wehmer; stalks colored as in *A. fumigatus*.....	*4651.2.*
Cultures of American origin.....	*4188.21*, *4138H52*, *4190.*
88d. Ascospores 4 by 4.5μ, red in acid, blue in alkali; sterigmata 9 to 18μ long. Probably incorrectly placed; Grijn's species. See 104h...	*A. fumigatus*, p. 129. = *S. pseudo-nidulans.*
88e. Ascospores globose 3 to 3.5μ.....	*A. fumigatoides*, p. 131.
88f. Related if not synonymous; organism of Eichelbaum.....	*A. virens*, p. 125.
89. Conidia described as globose (not ascigerous)	90.
89. Conidia described as subovoid.....	92.
90. Heads brown, in dense columns.....	93.
90. Heads green, not ascigerous, stalks colored, more or less sinuous.....	91. *A. fumigatus* series. 91a to n. Syn. *Eurotium fumigatum.*

Note: Costantin and Lucet propose to separate members of this series by their relative pathogenicity as tested upon laboratory animals. Recognition of the group is readily made; separation within the group is hardly practicable.

91a. Conidia 2.5μ; stalks 170 to 350μ long; vesicles 16 to 30μ diameter, not cultivated.....	*A. fumigatus*, p. 129.

91b. Colonies slow growing, forming a pseudoparenchymic felt..... *A. fumigatus* var. *tumescens*, p. 133.

91c. Conidia 2.5μ diameter, delicately spinulose; dark green colonies, greenish in reverse; culture from Neuberg received as..... *A. cellulosae*, p. 134. *4474.1.*

91d. Conidia 2.4 to 3μ; stalks arising from complex entanglements of hyphae..... *A. lignieresi*, p. 134.

91e. Conidia 2.5 to 3μ from soil by McBeth..... *2492.*

91f. Conidia 2.7μ from soil from Cuba..... *4149.*

91g. Conidia 3μ..... *A. pulmonum hominis*, p. 134.

91h. Conidia 2.8 to 3.6μ diameter; more or less floccose colony with a white sterile zone persisting for a considerable time at margin; stalks up to 400μ long..... *A. bronchialis*, p. 134.

91i. Conidia 2.8μ diameter; very floccose colony with persistent white areas; stalks up to 600μ long, often forked..... *A. virido-griseus*, p. 135.

Data enough to identify with *A. fumigatus* group but no ground for separation within the group.

91k. From inner costal surface of canary bird; not cultivated..... *A. aviarius*, p. 135.

91l. From the ear; group identification justified by figures..... *A. ramosus*, p. 135.

91m. Description inadequate, figure suggests probable synonymy and it is given as nearly related according to De Bary and Woronin 1866..... *A. nigrescens*, p. 135.

91n. Forms entirely unidentifiable but placed here on account of notes or fragmentary information.....
1. *A. glaucoides*, p. 136.
2. *A. microsporus*, p. 89.
3. *A. olivaceus* Preuss, p. 212.
4. *A. hageni*, p. 89.
5. *A. ageni*, p. 208.
6. *A. heterocephalus*, p. 210.
8. *A. quininae*, p. 213.

92. Conidia described as subovoid, otherwise corresponding with *A. fumigatus* group..... *A. syncephalis*, p. 136.

93. Heads brown, morphology of *A. fumigatus;* see also no. 143i..... *A. calyptratus*, p. 149.

93. Heads brown; mycelium brown to black; conidia 3 to 3.5μ, brown; pathogenic, see 143j..... *A. gratioti*, p. 150.

100. Heads with both primary and secondary sterigmata; heads with simple sterigmata found also in some forms (*A. jeanselmei* and *A. rehmii*). Occasional species or varieties described as not green. See No. 17..... 101.

101. Conidia 5μ or greater in long axis; conidia and secondary sterigmata greenish..... *S. chlorina*, p. 208.

101. Conidia less than 5μ (larger conidia occasionally found). Conidia mostly delicately spinulose (only occasionally apparently smooth)..... 102.

102. Perithecia described; a group of doubtfully separable ascosporic forms readily identified as a group but difficultly separable within the group..... 104a to k.

102. Perithecia not found..... 110.

104a. "Perithecia yellow with a greenish tone" produced in about 2 months; conidial areas light marine blue to chocolate brown; conidia 2.5 to 5μ finely "punctuated"..... *A. polychromus*, p. 137.

104b. Perithecia black surrounded by yellow mycelium; ascospores "smoke gray." See also 161a..... *A. rehmii*, p. 137.

104c. Perithecia more or less deeply enveloped in mycelium with swollen, very thick-walled sterile (Hülle) cells. Perithecial wall pink to deep red, almost black; stalks colored; conidial chains in columnar masses; ascospores purple..... *A. nidulans* group, p. 138.

104d. Asci 10.5 to 11μ diameter. Ascospores without band or frill. Asci developed slowly (several weeks)..... *A. nidulans*, p. 138.

Soil culture from Waksman reproducing Eidam's description..... *4163c28.*

104e. Variety pathogenic to man..... *A. nidulans* var. *nicollei*, p. 140.

104f. Asci 10 to 13μ diameter. Ascospores with 2 bands 1.5 to 1.8μ in width. Amsterdam strain..... *No. 110.*

104g. Ascospores with a single band, asci developed within a few days; sterigmata in 1 series 9 to 18μ long; Grijns' species..... *A. fumigatus*, p. 139.

104h. Ascospores with 2 bands..... *A. pseudo-nidulans*, p. 139.

104i. Ascospores with 2 bands 1μ or less in width. American soil strains..... *No. 131, 4110.*

104j. Ascospores with 2 bands, also ridges and folds on valves. New Jersey strain..... *4138T11.*

104k. Possible synonym: Species with very short stalks reddish brown; spores of the same color; perithecia seen on bread..... *S. purpurea*, p. 213.
110. Perithecia not found..... 111.
111. Conidial chains in solid columns..... 112.
111. Conidial chains radiating from a globose or subglobose vesicle..... 120.
112. Saprophytes..... 113a, b, c.
112. Parasites..... 114.
113a. Occasional strains differ from the above 104e to j series only in lacking perithecia..... *A. nidulans*, p. 138.
113b. Possible synonym: Suggested by the contrast described between a green color in the spores with a yellowish color described for the stalk..... *S. varia*, p. 213.
113c. Bainier in 1880 named three forms which he evidently had under observation at once. Neither of these presents the information required to determine whether the species belonged to the groups represented here by *A. nidulans*, *A. sydowi* or by *A. versicolor*, but they probably represent strains belonging in this section of the general group..... *S. prasina*, p. 213.
S. varia, p. 213.
S. minor, p. 211.
114. Parasites probably close to *A. nidulans*..... 115a, b, c.
115a. Not distinguishable by description from *A. nidulans* except as to parasitic habit upon the human nails, with conidia rough, 3μ diameter..... *S. unguis*, p. 140.
115b. With conidia smooth or rough up to 3.5μ, even variations up to 6.5μ..... *A. jeanselmei*, p. 140.
115c. Not positively identifiable: from the human ear..... *A. flavescens*, p. 141.
120. Conidial chains radiating from the vesicle or only occasionally forming a partial column. 121.
121. Colonies having the substrata uncolored or color not described..... 122a, b, c, d.
121. Colonies coloring the substratum..... 123.
122a. Colonies bluish green; variant strains of the group. See 124a..... *A. sydowi*, p. 147.
122b. Probably synonymous with 122a; stalk 140 by 4μ; heads globose; conidia smooth yellowish green, 3μ; see 127f..... *A. tiraboschii*, p. 148.
122c. Colonies dull green or yellowish green: variant strains of the group. See 124c, based upon.. *A. versicolor*, p. 142.
Represented by occasional strains in culture, such as..... *2712*.

122d. Colonies flesh colored or subochraceous; conidia 3 to 4μ; cell walls of stalk and conidia reddish; see 161b..... *S. spuria*, p. 147.

123. Colonies in reverse usually shades or tints of yellow, orange or red, often changing with the age of the colony or nature of the substratum; color sometimes tardily developing or even almost wanting..... 124.

124a. Colonies blue-green with reverse usually orange-red to blood red; with radiate heads; uncolored stalks 200 to 500μ by 5 to 7μ; conidia about 3 to 3.5μ, delicately spinulose; small heads, simple sterigmata and various combinations of sterigmata are found upon trailing aerial hyphae; a cosmopolitan group with numerous variable strains, many of which have been seen in culture. See also 122a..... *A. sydowi*, p. 147. *4725.1018, 3521. Kopeloff's 22.*

124b. Colonies emerald green, with red areas on wine casks; conidia smooth 4.2μ..... *S. glauca*, p. 145.

124c. Colonies in green, gray-green, buff and even flesh colored shades separately or, at times, several shades in the same colony; aerial mycelium often more or less colored; conidia 2.5 to 3.5μ or even 4μ, delicately few spinulose..... *A. versicolor group*, 125.

125. Conidial areas partly, at least, showing some shade of green..... 126.

125. Conidial areas without green color but cream, buff, flesh color, or almost rosy; otherwise *A. versicolor*..... 129.

126. Series of described species, apparently belonging in this group, but identifiable in culture with difficulty, if at all..... 127.

127a. Colonies* generally green varying through white, rose, salmon to brown; with stalks 300 to 350μ by 3.5 to 4.35μ; conidia 3 to 3.5μ.. *A. versicolor*, p. 142.

* Note: The following species are believed to be correctly placed in the group. It is not believed to be possible to identify cultures to particular species from the data given in the descriptions, but the recognition of the *A. versicolor* group is easily accomplished.

127b. Strain with minor differences in detail..... *S. polychroma*, p. 146.

127c. Colonies with range of characters fairly typical of this group..... *A. flavo-viridescens*, p. 146.

127d. Culture received from Hanzawa as *A. varians* and cited by him as synonymous with *A. flavo-viridescens*..... *4291.10.*

127e. Colonies in shades of yellowish, yellow-green, salmon and rose; with reverse in yellow and orange shades to dark brownish red.... *S. ambari*, p. 146.

127f. Possibly also: Colonies yellowish green; stalk 140 by 4μ; sterigmata primary about 5μ, secondary about 6.5μ long; conidia 3μ smooth, on sausage in Italy; compare 122b..... *A. tiraboschii*, p. 148.

127g. Colonies with margin in yellow shades, buff, etc., central area green..... *A. globosus*, p. 146. = *2705.*

127h. Colonies described with mycelium ochraceous; sterigmata containing red pigment; conidia 2.5μ, spinulose greenish; probably correctly placed here..... *A. bicolor*, p. 147.

127i. Colonies green with red color in the mycelium; conidia 3 to 3.5μ, spinulose. Obtained from an ulcerated hand, but pathogenicity not proved..... *S. tunetana*, p. 145.

127k. Colonies floccose, gray-yellow; stalks borne on aerial mycelium; see also *A. ustus* group.. *A. griseus*, p. 210.

Names.

127m. Cited by Waksman; apparently a typographical error for *A. versicolor*..... *A. diversicolor*, p. 147.

127n. Pathogen not placeable but probably in this section of the great group..... *A. pictor*, p. 90.

140. Conidial heads never green. See 16..... 141.

141a. Conidia thin-walled, smooth, or delicately spinulose, not pitted and not roughened with tubercles or bars of coloring matter; not brown or purple brown or black..... 142.

141b. Conidia roughened by definite tubercles or bars of color; mostly yellow to brown or to black..... 187.

141c. Conidia orange to umber in mass, thin-walled and almost smooth, or thicker walled and marked with pits, more or less irregular markings; orange to brown or umber shades, 4 by 5 to 5.5 by 6μ..... 215. The *A. wentii* group, p. 183.

142. Sterigmata in 1 series (arbitrarily placed here by the details of descriptions; cultures giving morphology and affinities not known)..... 143a to j.

142. Sterigmata in 2 series..... 150.
143a. Conidia 1μ in diameter, described as white... *A. pusillus*, p. 127.
143b. Conidia 2μ in diameter, described as white or greenish?..... *A. minimus*, p. 127.
143c. Conidia 3μ in diameter, cream yellow..... *A. koningi*, p. 127.
143d. Conidia 4 to 5μ (? really one series of sterigmata) (See *A. candidus* group); white..... *A. fimetarius*, p. 160.
143e. Heads pale yellow; conidia 2.5 to 3μ, smooth, pale yellow, compare 295..... *A. flavus* forma *maydis*, p. 203.
143f. Heads fawn color; conidia 2μ..... *A. cervinus*, p. 150.
143g. Heads fawn color; conidia 2.5 to 3.5μ..... *S. carnea*, p. 147.
143h. Possibly 143f seen in culture as..... *3522.36*.
143i. Heads avellaneus, described as black, figured as brown..... *A. calyptratus*, p. 149.
143j. Heads brown; mycelium brown to black; conidia 3 to 3.5μ, brown; pathogenic..... *A. gratioti*, p. 150.
143k. Heads subfuscus to olivaceus, stalks 1,000 by 15 to 20μ; conidia 3 to 3.5μ, smooth. Cited here on account of description, see 154d and 184g. *A. subfuscus*, p. 167.
150. Sterigmata in 2 series..... 151.
151. Conidial walls definitely spinulose..... 152.
151. Conidial walls smooth for with traces of spinulosity only)..... 155.
152. Stalk walls uniformly more or less deeply colored..... 154.
152. Stalk walls colorless or nearly so; colonies avellaneus; chains of conidia in columns.... *A. terreus*, p. 150.
152a. Probable synonym..... *A. fuscus*, p. 152.
152b. Pathogenic species, apparently close to *A. terreus*..... *S. hortai*, p. 152.
152c. Search in the literature for an early record of this form was fruitless except as to a description and figure closely resembling it in Corda Icones 3, p. 12, Taf. II, fig. 33, 1839, as.... *Rhodocephalus aureus*, p. 152.
152d. Possible identity suggested by figure but not by description; see 143i..... *A. calyptratus*, p. 149.
154a. Colonies gray or olive toward fuscous (not truly green although greenish in effect at times). Walls of stalks and foot-cells some shade of olive grey. Conidia 3 to 4μ, outer wall more or less reddish or brownish, spinulose; colonies mostly showing floccose felts of aerial hyphae; heads radiate; stalks mostly short branches and from aerial networks of hyphae, occasionally arising from the substratum and very long..... *A. ustus*, p. 152.
Among many cultures placed here..... *4640.488*. *3556*.

154b. Colonies floccose, gray-yellow, with stalks borne as branches of aerial hyphae; possibly this group..... *A. griseus*, p. 210.

154c. Conidia up to 5μ, much darker than 154a with coloring matter sometimes showing as tubercles or bars on conidial walls; colonies almost fuscous..... *A. insuetus*, p. 153.
Description apparently satisfied by..... *4658.245.* *4303.65.*

154d. Heads subfuscous to olivaceous; conidia 3 to 3.5μ smooth (?)..... *A. subfuscus*, p. 167.

155. Conidial walls smooth (? or nearly so)..... 156.

156. Stalk walls uniformly yellowed, especially in outer layer; heads white or tardily faintly yellowish: chains of conidia in columns.... *A. flavipes* group, 157.

156. Stalk walls not colored (or only slightly colored at apex)..... 165.

157. Perithecia not found; sclerotia and mycelial crusts found in some strains..... 158a to d.

157. Perithecia reported..... 160.

158a. Stalks 300 to 400 by 3 to 4μ, conidia 2 to 3μ, colorless..... *A. flavipes*, p. 155. *4640.474, 4640.402, 4118.4, 4291.4.*

158b. Stalks up to 2,000 by 7 to 10μ; aerial tufts of bright yellow mycelium but no perithecia; sclerotia and mycelial crusts or stromata brown to almost black..... *4777.16, 2713.*

158c. Could this be..... *S. albo-lutea*, p. 208.

158d. Possibly placed here on account of colonies white and "stalks sublexuous," but otherwise unknown..... *A. mülleri*, p. 212.

160. Perithecia found..... 161.

161a. Perithecia black surrounded by a fairly dense yellow mycelial felt (Hülle). See 104b..... *A. rehmii*, p. 137.

161b. Possibly: stalk walls colorless to reddish brown; stalks up to 500 by 10μ; conidia 3 to 4μ. See 122d. (See also *A. versicolor* group.)..... *S. spuria*, p. 147.

161c. Suggested as possibly related from descriptions; compare 143g..... *S. carnea*, p. 147.

165. Stalk walls smooth, colorless (or slightly colored near the vesicle)..... 166.

166. Heads white or pale cream yellowish in some forms, especially in very old cultures..... *A. candidus* group, 167 to 178.

166. Heads yellow, ocher, cinnamon or approaching fuscous..... 180.

167. Stalks commonly or partly borne as branches of anastomosing ropes of aerial hyphae..... *4658.54/3.*

167a.	Compare 184d.....Possibly	*S. veneta*, p. 163.
167.	Stalks rising mostly from submerged hyphae.	168.
168.	Either ascospores or sclerotia reported.....	178.
168.	Ascospores and sclerotia not reported.....	169.
169.	Conidia globose.....	170.
169.	Conidia more or less definitely elliptical or ovate.....	176.
170.	Conidial measurements not given.....	*A. candidus* Link, p. 157.
170a.	Cited by Link as.....	*Monilia candida*, p. 157.
170.	Conidial measurements more or less definitely given.....	171.
171.	Conidia 1.5 to 2μ; secondary sterigmata 10 to 12μ in a crown at the apex of each primary sterigma; heads 1 mm. in diameter.....	*S. coronata*, p. 159.
171.	Conidia larger than 2μ.....	172.
172.	Conidia 2.5 to 3.5μ; stalks described as slender, less than 10μ in diameter.....	173a, b, c.
172.	Stalks coarser.....	174.
173a.	Conidia 2.7 to 3.5μ; stalk about 7μ diameter..	*A. albus* Wilhelm, p. 159.
173b.	Conidia 3.2μ; stalk up to 8.4μ diameter; primary sterigmata up to 16.8μ long.....	*S. alba* Bainier, p. 158.
173c.	Conidia 3.1μ; stalk up to 6.3μ diameter; sterigmata primary 4.2μ, secondary 10.5μ long; Bainier's.....	*S. candida*, p. 159.
174.	Conidia varying from 2.5 to 5μ; stalks coarser, up to 15μ in diameter.....	175a to g.
175a.	Conidia 2.5 to 3.5μ; stalks 11 to 15μ by 1 to 2 mm.; sterigmata primary 7 to 10μ, secondary 7 to 10μ long; Saccardo's.....	*S. candida*, p. 158.
175c.	Conidia 2.8 to 3.5μ or even 4μ; stalks 500 to 700 by 10 to 12μ to 2,000 by 15μ or more; sterigmata primary 5 to 7 to 10 even to 20μ, secondary 5 to 6μ long.....	*No. 106.*
175d.	Conidia 3 to 3.5μ; primary sterigmata 32 to 40 by 10μ; stalks 1 to 2 mm. by 16 to 17μ.....	*S. szurakiana*, p. 162.
175e.	Conidia 2.5 to 5μ, cream yellow in age; stalks smooth or rough, warty, 5 to 13.5μ by 150 to 550μ; sterigmata primary 12 to 20μ, secondary 6 to 14μ long.....	*A. okazakii*,* p. 157.

* Biochemical reactions were developed by Okazaki to separate this from two other forms regarded by him as *A. albus* and *A. candidus*. The "Ferri-chloride" separation of *A. okazakii* taken with the rough stalk described suggests a different relationship for this organism than that indicated by Takahashi's culture or by Okazaki's paper.

175e. Conidia 3μ; stalk 150 to 250μ, even to 350 by 8 to 12μ; sterigmata primary 7 to 9μ, secondary 7 to 9μ long; culture from Takahashi as.. *A. okazakii.*

Questionable white forms.

175f. Conidia 4μ, asperulate; colonies white to sulfur yellow; heads large; compare 203c..... *S. ochroleuca*, p. 186.

175g. Conidia 4 to 5μ; one series of sterigmata described..... *A. fimetarius*, p. 160.

176. Conidia more or less elliptical or ovate..... 177.

177. Conidia 5 by 3μ, vesicle 40 to 50μ, fertile on upper part only; stalks up to 16 to 18μ diameter (not cultivated)..... *S. coronella*, p. 159.

177. Conidia ovate, rather thick-walled, with yellowish contents, stalk pellucid, sterigmata 1 series, vesicle cut off by a septum; colonies designated white..... *A. dubius*, p. 160.

178. Sclerotia observed in culture.

178a. Sclerotia purple, stalks up to 500μ long, conidia globose 3 to 3.5μ..... *4126.1.*
4123.1.
4781.

178. Ascosporic form..... *A. albidus*, p. 162.

180. Heads yellow to ocher yellow. The real affinities of this series of species appears to be with the *A. ochraceus* group; see no. 223..... 181.

180. Heads cinnamon, fuscous to almost black.... 184f, g, h.

181. Sclerotia observed..... 182.

181. Sclerotia not reported..... 183.

182. Sclerotia white to black abundant with heads few and scattered, stalks smooth, and conidia 2.5 by 3μ, or less commonly 3μ..... *4660, 4656. A. alliaceus*, p. 163.

182. With stalks up to 10 mm. long by 20μ diameter; heads large; conidia globose, smooth 4 to 4.3μ..... *S. quercina*, p. 186.

183. Sclerotia not reported: An arbitrary series probably not all related; separated by color, spore measurement, etc..... 184.

184a. Heads sulphur yellow; stalks colorless, smooth; primary sterigmata long, in compact radiate series; conidia globose about 2.5 to 3.5μ figured as smooth; frequently referred to *A. ochraceus* group..... *A. sulphureus*, p. 185.

184b. Measurements as in *A. sulphureus* except conidia asperulate 4μ..... *S. ochroleuca*, p. 186.

184c. Heads ocher yellow..... *S. ochracea*, p. 163.

184d. Heads pale yellowish; stalks "fasciculate;" sterigmata primary 4 to 6 by 2 to 3μ, second-

ary 6 to 8 by 2μ; conidia 2 to 3μ, scarcely yellow, globose. Compare 167a..... *S. veneta*, p. 163.

184e. Differing in conidia 5 to 6μ diameter, verrucose..... *A. corolligena*, p. 164.

184f. Heads pale cinnamon, conidial walls diffusely faintly colored; otherwise *A. niger* from which it is described as a mutant..... *A. cinnamomeus*, p. 164.
Schiemann's culture studied as..... *3534b*.

184g. Heads subfuscus to almost black, secondary sterigmata more or less suppressed..... *A. subfuscus*, p. 167.

184h. Heads black or in black brown shades..... 185 to 211. The *A. niger* group.

185. Conidia smooth or nearly so, color diffused, not as tubercles or color bars..... 186.

185. Conidia tuberculate with masses or bars of color..... 187a, b, c.

186a. Conidia thin-walled, smooth 3.5 to 4.5μ in diameter, color diffused, not aggregated into tubercles or bars; received from Neuberg as *A. niger citricus*..... *4668.4*.

186b. Conidia smooth, 4.2μ in diameter, with primary sterigmata about 8.4μ long..... *S. faliginosa*, p. 166.

187a. Conidia about 5μ in diameter; rough with tubercles and color bars when observed dry but which break up and disappear in fluid mounts (alcohol, water, etc.), leaving conidia smooth, thin-walled, otherwise belonging with *A. niger*..... *4714* and *4729*.

187b. Smooth spores as found in mounts from *4714* and *4729* satisfy description of conidia of... *A. luteo-niger*, p. 166.

187c. Closely related culture, found in Bainier's collection labeled as..... *4640.482*. *S. pseudo-carbonaria*, p. 166.

187d. Conidia roughened by persistent color bars or tubercles of coloring matter mostly globose but in a few forms showing ellipticity; colonies reach some shade of brown to black from colorless (through definite yellow only in no. 203b). Compare 187a, b, c; also no. 261, *A. japonicus* with rough stalk wall..... 188.

188. Sterigmata in 1 series only..... 189.

188. Sterigmata in 2 series..... 196.

189. Sclerotia not reported..... 190a, b, c, d, e.

189. Sclerotia found..... 191.

190a. Black heads enveloped in slime; stalks also black..... *A. echinosporus*, p. 209.

190b. Sterigmata about 6 by 3μ; conidia 3.5μ rarely 4μ, color distributed as spines or spiny tubercles rather than bars..... *4202.18c* and *N.0.3*.

These satisfy the description of.....	*A. luchuensis*, p. 171.
190c. Hanzawa's culture received under this name has partly double sterigmata.....	*4291.3.*
190d. Sterigmata shorter than in *A. luchuensis*, heads and conidia white to yellow to yellow brown; conidia 4 to 5μ rough.....	*A. perniciosus*, p. 171.
190e. Sterigmata up to 15μ in length; conidia 3μ; mycelium "flavescent".....	*A. nanus* Montague, p. 170.
Not	*A. nanus* Oud.
191. Sclerotia abundant. Sterigmata 1 series.....	192.
192. Conidia mostly elliptical or tardily partly globose.....	193.
192. Conidia mostly globose.....	195.
193. Conidia elliptical 3 to 4 by 4 to 5μ.....	194.
194a. Culture sepia to chocolate brown; conidia 3.5 by 5μ, more delicately marked.....	*4725.688.* *3522.30.*
194b. 194a compares closely with the description of.....	*A. violaceo-fuscus*, p. 172.
194c. Aberrant form with superficial resemblances to these species. Fonseca contributes a yellow brown culture with a transient greenish tinge; conidia 4 to 8μ in diameter have color bars yellow arranged as in *A. niger*, but careful study of its sinuous stalks shows traces of the pitting like *A. tamarii* (See no. 279a).....	*4707.757.*
195. Heads almost black; stalks 400 to 700 by 12 to 14μ, colorless or yellowed at top; reverse of colony golden yellow; sterigmata 12 to 13 by up to 4 to 5μ; conidia 3 to 4.5μ, mostly 3.5 to 4μ, spiny.....	*4030.3.* *4030.5.*
195a. *4030.3* and *4030.5* possibly represent.....	*A. japonicus*, p. 171.
196. Sterigmata in 2 series, primary and secondary.	197.
197. Conidia 2.5 to 4μ (occasionally up to 5μ). Mature conidia rough.....	198.
197. Conidia larger.....	207.
198. Primary sterigmata usually 20 to 30μ long in mature average sized heads in culture media.....	199.
198. Primary sterigmata much longer.....	204 a to g.
199. Colonies pale fuscous.....	200a, b, c.
199. Colonies in other black brown shades.....	201.
200a. Conidial markings faint but present; morphology otherwise as *A. niger*.....	*A. schiemanni*, p. 172.

200b. Hanzawa has contributed an apparently synonymous culture without description recorded as..... *4291.2. A. awamori*, p. 173.

200c. A culture with stalks rather thin-walled; color diffused through vesicle, and sterigmata, with narrow bars on the conidia..... *4727.5.* *4755.*

201. Colonies in yellow and brown rather than black shades..... 203.

201. Colonies brown, purple brown to black (variant lighter shades occasionally found with obvious relationship by structure); conidial roughening evident..... 202. *A. niger* series.

202a. Conidia globose mostly less than 4μ in diameter..... *A. niger*, p. 167.

202b. Forms certainly belonging in a group with *A. niger* but not separable by morphological data in the descriptions given..... 1. *A. fuliginosus*, p. 174.
2. *A. nigriceps*, p. 175.
3. *S. pseudonigra*, p. 175.
4. *A. ficuum*, p. 174.

202c. Forms probably belonging with *A. niger* but positively unidentifiable..... *A. pulla*, p. 168.
A. nigrescens, p. 135.
A. nigricans, p. 174.
A. mucoroideus, p. 211.
A. fuscus, p. 210.
A. strychni, p. 178.

203a. Conidia globose 4 to 5μ, brown rather than black, otherwise as *A. niger*..... *S. castanea*, p. 174.

203b. Colonies reaching blackish brown through yellow; primary sterigmata 24 to 40 by 8μ... *A. batatae*, p. 173.

203c. Conidia globose 4μ, asperulate, white to sulfur yellow, measurements of *A. niger*. Probably elsewhere, see no. 175f..... *S. ochroleuca*, p. 186.

203d. Conidia globose 4 to 8μ with markings yellow; in mass brown as in *A. tamarii*, stalk with trace of pitting..... *4707.757.*

204. Primary sterigmata up to 50 to 60μ long..... 205a, b, c, d, e, f.

204. Primary sterigmata much longer than 50μ.... 206.

205a. Primary sterigmata varying to about 50μ long *A. phoenicis*, p. 175.

205b. Primary sterigmata 60μ long..... *A. phaeocephalus*, p. 177.

205c. Primary sterigmata 40 to 50μ long; conidia 4 to 4.5μ..... *Aspergillopsis intermedia*, p. 177.

205d. Primary sterigmata 40μ, conidia very black 4 to 5μ..... *S. longobasidia*, p. 177. *4640.477.*

205e. Primary sterigmata 46μ.....	*S. nigra* Bainier, p. 177.
205f. Primary sterigmata 50μ, secondary sterigmata not described, conidia 2.5 to 3.9μ.....	*A. ustilago*, p. 177.
205g. Primary sterigmata 20 to 50μ.....	*S. antacustica*, p. 176.
206. Primary sterigmata very long, up to 120μ.....	*A. pulverulenta*, p. 179. *A. strychni*, p. 178. *S. welwitschiae*, p. 178.
207. Conidia with long axis more than 5μ.....	208.
208. Primary sterigmata ordinarily less than 40μ in long axis.....	209.
208. Primary sterigmata much longer.....	210.
209a. Conidia 7 to 8.5μ in diameter, otherwise as in *A. niger*.....	*S. dipus*, p. 180.
209b. Probably synonymous ..... Not	*S. fusca* Bainier, p. 180. *A. fuscus* Bonorden, p. 210.
209c. Conidia described as 6 to 8μ purple black.....	*A. atropurpureus*, p. 179.
209d. Fonseca's Brazilian culture with conidia 5 to 10μ in diameter satisfies the description of *S. fusca* Bainier.....	*4707.878.*
209e. Conidia 5μ (up to 8μ in old cultures) in long axis, with color bars lengthwise; yellow-brown heads few, large, upon abundant sterile mycelium; culture from Neuberg labeled	*4668.2. A. fumaricus*, p. 181.
209f. Possibly an intermediate: Conidia 8 to 10μ...	*Aspergillopsis pulchella*, p. 181.
210. Primary sterigmata much longer.....	211.
211. Conidia 8 to 10μ in diameter, heads carbonaceous, black, primary sterigmata up to 120μ long and carbonaceous.....	*A. carbonarius*, p. 181. *4030.1.*
215. Conidia pitted or grooved, or smooth and thin-walled, not roughened; coarse long stalked, with heads orange yellow to brown or umber. See 141c.....	216.
216. Conidia pitted or smooth, pyriform to globose, 4 by 5μ, to 5.5 by 6μ; colonies brown.....	*A. wentii*, p. 183.
216a. Culture: A variety, (?), very thin-walled.....	*4700.A32.*
216. Conidia globose up to 4.2μ, color bronze (aereus).....	*S. aerea*, p. 184.
220. Stalks pitted, sometimes also granular, often reported as rough or spinulose when observed with low magnifications; pitting only partially detectable in some forms (See 15)..	221.
221. Colonies never green (verging into greenish bronze in transition forms at end of group)..	222.
221. Colonies some shade of green or yellow-green on favorable media.....	290.

222.	Conidia small, 2 to 5μ (individual cells rarely larger) in long axis; mostly less than 4μ (no record in *A. fulvus*)	223.
222.	Conidia greater than 5μ in long axis; 254h and i.	265.
223.	Stalks colorless	260.
223.	Stalks yellowed, commonly showing surface concretions or warts; yellow color mostly in the outer layers of the stalk-wall, but in a few strains both the color and pitting of the wall is almost suppressed	224.
224.	Conidial heads in fairly pure yellow colors, sulphureus, citrinus or flavus of Saccardo's chromotaxia, Code de Couleurs 156, 161, 216, 221 or about Ridgway Plate XV, 15′, and XVI, 23′	225. *A. sulphureus** series.
224.	Conidial heads in shades of cremeus, ochroleucus, ochraceous, or melleus (Saccardo), or Code de Couleurs 146, 141, 162, or about Ridgway Pl. XXX, 19″	240. *A. ochraceus** series.
	* Note: While these two series are closely related and may be difficult to separate the contrast between the bright yellow series and the ochraceous or buff series is commonly very marked.	
225.	Sclerotia not found	226.
225.	Sclerotia reported, yellow forms	229.
226.	Without sclerotia. Stalks described as colorless smooth	227a and b.
226.	Stalks yellow and pitted	228.
227a.	Heads sulfur yellow; conidia globose 2.5 to 3.5μ, figured as smooth; compare 184a	*A. sulphureus*, p. 185.
227b.	Description of *A. sulphureus* except conidia 4μ, minutely asperulate; compare 184b	*S. ochroleuca*, p. 186.
228.	Colonies floccose in center with marginal yellow heads; stalks up to 300 by 4 to 6μ, conidia 2.5 to 3μ	*4700A34.*
228a.	Pathogenic; heads golden yellow to "brick red," otherwise as in 228	*S. aurea*, p. 85.
229.	Sclerotia reported	230.
230.	Stalks apparently smooth, colorless (or pitting demonstrated with difficulty)	231a and b.
230.	Stalks yellow-pitted	232a, b, c.
231a.	Sclerotia abundant with heads few and scattered, stalks short, smooth colorless; conidia 2.5 by 3μ, less commonly 3μ and globose (compare 182)	*4660, 4656. A. alliaceus*, p. 163.

231b. Sclerotia very abundant on carrot; stalks up to 10 mm. by up to 20μ in diameter; heads large; conidia globose, smooth 4 to 4.3μ..... *A. quercinus*, p. 186.

232a. Colonies as in *S. quercina*, except that stalks up to 4 to 5 mm. long which appear almost smooth and colorless at first appear pitted and with outer layer yellow in older cultures; conidia smooth, ovate 3 to 4μ in long axis.. *4754c76.* *4186.1.* *4078K2.*

232b. Stalks 460 to 1,000μ by 5 to 12μ, either smooth or rough; conidia globose, finely spinulose, 2 to 4.6μ..... *A. sulphureus* var. unnamed Kita.

232c. Stalks about 1,000 by 10 to 14μ, part of secondary sterigmata grown out into short orange hyphae; conidia 3.5 by 3.8μ with walls thick, yellow..... *S. auricoma*, p. 187.

240. Conidial heads in shades of cremeus, ochroleucus, ochraceous and melleus..... 241. *A. ochraceus* series.

241. Sclerotia reported..... 250.

241. Sclerotia not reported..... 242.

242. Stalks apparently smooth or pitting demonstrable only with great care..... 243.

242. Stalks yellow and pitted..... 244.

243. Stalks "uncolored smooth;" heads globose; sterigmata primary 10.5μ, secondary 6.3μ; conidia 3.2μ (compare 184c)..... *S. ochracea* Bain., p. 163.

244. Conidia finely spinulose or verruculose..... 246.

244. Conidia smooth, thin-walled..... 245a to f.

245a. Conidia smooth, thin-walled, 2.5μ to 3μ not larger; stalks sinuous; sterigmata primary 6 to 8μ, secondary 7 to 8μ in length..... *4218.2.*

245b. Dwarf, light ochraceous buff, from Japan; stalks less than 500μ; conidia 3 to 4μ; with color in substratum almost red and occasionally magenta in vesicles and sterigmata..... *4700a* and *c.*

245c. Conidia smooth, thin-walled up to 3μ; colonies brownish purple below..... *4725.475.*

245d. Conidia small, up to 3.5μ; heads globose, regular or nearly so..... *112. 4718.*

245e. Conidia small, up to 3.5μ; heads large splitting into several solid columns; mycelial aggregations seen but no sclerotia..... *4399.*

245f. Conidia 3.5 to 4μ hyaline; stalks 500 to 600 by 12μ (Czecho Slovakia)..... *4652.*

246a. Conidia spinulose small, up to about 3.5μ. Exsiccati..... *Langlois 1888.*

246b Conidia 3.5 to 4μ or 3.5 by 4 to 4.5μ rough; (Holland) ..... *4251.*

246c. Conidia 3 to 4 by 4 to 5μ rough, otherwise close to Wilhelm's description but without sclerotia (culture lost) ..... *Metheny's no. 2.*

250. Sclerotia reported 251

251. Conidia smooth ..... 252a to f.

251. Conidia spinulose or verruculose ..... 253.

252a. Stalks and yellow sclerotia both abundant. Stalks 3 to 5 mm. by 12 to 15μ, conidia up to 3.5μ in long axis; reverse more or less deeply yellowed ..... *4658.80/2.*

252b. Sclerotia vinaceous, otherwise as in 252a ..... *4754.c77.*

252c. Pinkish sclerotia abundant; stalks few, scattered; heads pale buff; conidia up to 3μ .... *4725.806.*

252d. Stalks up to 1,000 by 7 to 9μ; conidia 2.5 to 3.6μ, few and scattered among sclerotia .... *4138n43.* *4741.*

252e. Conidia 2.5 to 4μ; colonies white to honey yellow; sclerotia 0.4 to 0.7 mm. yellowish brown ..... *A. melleus*, p. 188.

252f. *A. melleus* is fairly represented by culture from Hanzawa ..... *4291.6.*

253. Conidia spinulose or verruculose ..... 254a to j.

254a. Conidia 3 to 3.5μ, stalks slender ..... *A. elegans*, p. 189.

254b. Culture close to 254a received from Raistrick as *A. elegans* (1924) ..... *4724.40.*

254c. Conidia 3.5 to 5μ in long axis, conidial heads abundant with sclerotia fairly abundant on surface of substratum; conidia 3.5 by 5μ, or 3 to 4μ, even to 4.5μ diameter ..... *A. ochraceus* Wilhelm, p. 188.

254d. Wilhelm's exsiccati ..... *Rabh. F. Europaei* no. 2361.

254e. Conidia 3 to 3.5μ or 3 by 3.5μ, in large heads conspicuously splitting into bundles. Sclerotia very abundant ..... *2399.*

254f. Conidia 2.5 to 4.2μ, slightly elliptical; colonies tan; on corn ..... *A. alutaceus*, p. 189.

254g. Colonies with the aspect of *A. ochraceus*, but conidia 7 to 8μ in diameter and warty, compare 271 ..... *S. delacroixii* Sacc., p. 190.

254h. Conidia somewhat pyriform, about 3 to 4μ by 5μ in long axis, in age up to 4.5 by 6μ, with wall bearing delicate color bars; received from Raistrick as *A. ostianus* ..... *4724.35.*

254i. Conidia "mostly smooth," yellowish, 4 to 5μ.. *A. ostianus*, p. 190.

260. Stalks colorless (compare 223) (very pale yellowish to almost colorless stalks are found in some members of the preceding group... 261.

261. Conidia 4 to 5μ; "blackish brown," rough; heads blackish brown; sterigmata 1 series; sclerotia produced (compare 195a)..... *A. japonicus*, p. 171.

261. Conidia smooth, measurements not given; colonies fulvous; sterigmata in 2 series; upon silkworms..... *A. fulvus*, p. 195.

265. Conidia more than 5μ in long axis..... 266.

266. Conidia roughened with spinules, tubercles, or bars of coloring substance..... 270.

266. Conidia smooth (compare *A. fulvus* no. 261).. 267.

267a. Conidia described as commonly smooth; compare no. 254i..... *A. ostianus*, p. 190.

267b. Conidia smooth 5.2μ as described (up to 6.2μ and rough in exsiccati)..... *S. butyracea*, p. 191.

267c. Conidia up to 8 to 12μ or larger, variable, smooth; colonies yellow brown; stalks 300 to 780μ by 18 to 20μ; probably more correctly placed at 23i in the *A. glaucus* group..... *A. godfrini*, p. 109.

270. Conidia finely warty or roughened with tubercles or bars of color..... 271.

271. Stalks yellow, pitted; conidia 7 to 8μ, finely warty, otherwise as in *A. ochraceus*. See 254g..... *S. delacroixii* Sacc., p. 190.

271. Stalks not yellow..... 272.

272. Sterigmata in 1 series..... 273.

272. Sterigmata in 1 series or in 2 series in same culture..... 275.

273. Perithecia not reported..... 274a to d.

273a. Perithecia reported but not obtained readily in culture; conidia 5 to 9 by 5 to 6μ, lemon-shaped, beautifully marked with delicate yellow bars of color; heads golden when young, brown in age..... *A. citrisporus*, p. 191.

273b. Cultures apparently representing this species. *4186.10, 4394, 4730, 4301.10.*

273c. Possible synonymy, based upon golden yellow elliptical conidia..... *A. aureus*, p. 216.

274a. Vesicles 40 to 50μ; conidia umbrinus, measurements not recorded..... *A. terricola*, p. 192.

274b. Probable synonym: Conidia 5 by 7μ, occasionally 8 to 9μ, subglobose, tuberculate with yellow or rusty coloring matter..... *A. lutescens*, p. 193. *4640.478.*

274c. Possible synonym..... *A. fimeti*, p. 209.

274d. Vesicle smaller than *A. terricola*, and conidia less prominently roughened; colonies rusty to umber	*A. terricola* var. *americana*, p. 192.
275. Sterigmata in either one or two series in same colony and often in the same head	276.
276. Colonies at first orange yellow then deep brown; conidia pyriform to globose 5 to 8μ in long axis, roughened with rather coarse bars of brown coloring matter; stalks usually thick-walled occasionally with pitting inconspicuous or partly suppressed; cosmopolitan; variable; abundant	*A. tamarii*, p. 194.
The following forms all belong with *A. tamarii* in fairly close relationship	277a to g.
276. Colonies approximating *A. tamarii* but with greenish brown or bronze tones	278.
276. Colonies in colors of *A. tamarii* group but much larger conidia	280.
277a. Colonies umbrinus from Brazil nuts	*A. umbrinus*, p. 195.
277b. Culture of *A. tamarii* from similar situation	*4735*.
277c. Culture *4640.397* from Bainier's collection marked	*A. cacao*, p. 196.
277d. Probably the same strain (277c) distributed by Pribram	*4777.4*.
277e. Colonies orange to chocolate-brown; conidia 5.5 to 6μ (according to Sartory and Jourde)	*S. fusca* Bain., p. 180.
277f. Conidia 5 to 6μ; smooth or delicately roughened; colonies pale brick red (testaceus); a very large form	*A. gigas*, p. 196.
277g. From deteriorating sugar; description indicates *A. tamarii*	*A. spadix*, p. 195.
278. Colonies described with more or less green or greenish color or effect; conidia roughened with color-bars	279.
279a. Colonies with green tinge at first; then quickly bronze or brown; conidia 4 to 8μ, markings of *A. niger* but color of *A. tamarii;* pitting of stalk present but only on very careful examination	*4707.757*.
279b. Colonies green on Czapek with yellow mature conidia 5 to 7μ in long axis; short stalked *A. flavus*-like but with rough spores	*4725.248*.
279c. Conidia 5 to 8μ; stalk 500μ long; head 350μ diameter	*4795*.
279d. Conidia green: coarsely roughened with color bars; sclerotia with white tips	*4417.3*.
280. Conidia larger than *A. tamarii*, elliptical, pitted rather than tuberculate	282.

280. Forms described with the colors of the *A. tamarii* group but with larger conidia and suggesting conidial numbers of the *A. glaucus* group with the green of the conidial head masked by the general colony color..... 281a to d.

281a. Conidia 9 to 10μ orange yellow shades..... *A. sartoryi*, p. 122.

281b. Conidia 10 to 12 by 10μ rough, colonies greenish (? bronze) to brick red. See also 51e..... *A. rufescens*, p. 121.

281c. Possibly (?) conidia 12 to 18 by 8 to 15μ, echinulate; colonies bronze. (See also 51a in *A. glaucus* group.)..... *A. brunneo-fuscus*, p. 117.

281d. Conidia 15 to 18 by 12 to 42μ (33 by 21μ and larger in chloral hydrate preparation). See 51d *A. glaucus* group..... *A. ochraceo-ruber*, p. 120.

282. Conidia pitted rather than tuberculate with color masses..... 283.

283. Gigantic forms..... 284.

284a. Form with aspect of *A. ochraceus* group; conidia elliptical 8 to 12μ by 5 to 8μ, yellowish, pitted; stalks about 10 mm. or longer by 50 to 70μ diameter; primary sterigmata up to 50 to 120μ long; secondary 15 to 25μ..... *A. penicillopsis*, p. 196.

284b. Form with heads red or reddish; conidia 10 to 12 by 6 to 9μ..... *A. erythrocephalus*, p. 197.

290. Colonies* mostly some shade of green or yellow green or greenish yellow. Some strains lose the green color in age; some appear to lack green color but to have the same underlying shades of yellow as in the group. Conidia without color bars often almost smooth at first; usually marked with more or less winding pits giving frequently a falsely rough appearance when studied with low magnifications. No separation of primary (outer) from secondary (inner) conidial wall is visible (compare 276 also)..... The *A. flavus-oryzae* group, 291 to 307.

*Note: Hundreds of cultures have been seen and studied in this group; variations bridging the gaps between the species named have been seen in nearly every section of this group. Naturally many species have been made by various authors from material apparently representing the group but not sufficiently described to make separation possible.

291. Colonies with stalks arising mostly from the substratum, not from floccose aerial mycelium..... 292.

291. Colonies with floccose mycelium, and with stalks borne mainly as short branches; green color often nearly suppressed..... 307.

292. Sterigmata described in 1 series; secondary sterigmata are usually occasionally found even in type cultures but are not the rule... 293.

292. Sterigmata partly simple but commonly in 2 series in the same heads or in different heads of the same colony..... 296.

293. Green color in heads definite, abundant..... 294a and b.

293. Green color faint, often almost or completely suppressed..... 295.

294a. Colonies near ivy green (fairly deep green); stalks 200 to 400μ long; conidia 3 to 5 or even 6μ, mostly 3 to 5μ, on mealy bugs..... *A. parasiticus*, p. 203.

294b. Type culture from Speare..... *3509*.
Contributed also by Kopeloff (*no. 21*) from Louisiana, and from Barbados by J. R. Johnston.

295. Colonies pale yellow (no green color reported); conidia 2.5 to 3μ smooth (?). Compare 143e..... *A. flavus* forma *maydis*, p. 203.

295. Stalks long, loosely developed; sterigmata described as simple but occasional branched forms found..... *A. oryzae*, p. 198.

296. Sterigmata in 2 series. Probably inadequate for separation from 295..... *A. oryzae* var. *basidiferens*, p. 199.

296. Sterigmata often partly 1 series, partly 2 series in the same heads, especially the larger heads..... 297.

297. Sclerotia not reported..... 298.

297. Sclerotia reported..... 305.

298. Saprophytes..... 299.

298. Parasites to warm blooded animals..... 303.

299. Stalks commonly less than 1,000μ in length... 300 a to d.

299. Stalks commonly 1 to 2 mm. long..... 301.

300a. Colonies fairly green (Krönberg's green on sugar media); stalks usually 400 to 700μ long, rarely 1,000; conidia pyriform to globose varying from 2 by 3μ to 5 by 6μ or longer.. *108* and *3526*.

300b. 300a was regarded by Wehmer as..... *A. flavus*, p. 198.

300c. The same material was characterized as n.sp. by Costantin and Lucet..... *A. wehmeri*, p. 204.

300d. Colonies with about same characters..... *A. variabilis*, p. 204.
301. Stalks 1 to 2 mm. long..... 302 and b.
302a. Stalks 1 to 2.5 mm. long by 10 to 20μ diameter, "with rough warty walls;" yellowish-green, "leaf"-green to dark brown in very old cultures; conidia 4 to 6μ..... *A. gymnosardae*, p. 204.
302b. Stalks 1 to 2 mm. long; colonies yellow-green; conidia mostly 6 to 7μ, varying from 5 to 8μ diameter..... *A. pseudo-flavus*, p. 205.
303. Pathogenic..... 304.
304a. Reported from the human ear but not definitely identified..... *A. flavescens*, p. 141.
A. nölting, p. 205.
304b. Stalks 600 to 1,700μ; otherwise *A. flavus*..... *A. micro-virido-citrinus*, p. 205.
304c. Stalks up to 4 mm. long; renaming of *A. flavus* of Siebenmann..... *A. siebenmanni*, p. 206.
305. Sclerotia reported..... 306.
306a. Wilhelm adds the observation of "sclerotia minuta nigra tuberosa" to the citation of Brefeld's culture in Rabenhort, Fung. eur., Edit. Nov., Ser. II. 2135..... *A. flavus*, p. 198.
306b. This material (seen by us) corresponds closely to cultures (see 300a)..... *108* and *3526*.
306c. De Bary and Woronin transferred the species to *Eurotium*..... *E. aspergillus flavus*.
307a. Colonies with floccose mycelium with stalks mainly borne as short branches; stalks reduced greatly, footcells often very long and only partly differentiated from vegetative cells of hyphae..... *A. effusus*, p. 206.
Our conception based upon cultures..... No. *130*, *Sc171*, *2750*.
307b. Colonies described as a beautiful yellow, greenish in age; with heavy masses of aerial mycelium; conidia globose, smooth 6.4μ. Culture *4640.473* under this name in the Bainier Collection was *A. flavus*..... *S. lutea*, p. 207.
307c. Colonies on cheese white with conidial cell-contents yellowish and stalks "pellucid" (pitted or roughened)..... *A. dubius* Corda, p. 160.
307d. Conidia 6μ, smooth or nearly so. Noted as near *A. dubius*..... *S. italica*, p. 161.

XVI. ACCEPTED SPECIES[1]

A. ALLIACEUS n. sp., p. 163.
A. AMSTELODAMI (Mangin), p. 113.
A. ATROPURPUREUS Zimmermann, A., p. 179.
A. CANDIDUS Link, p. 157.
A. CARBONARIUS (Bainier) Thom, p. 181.
A. CHEVALIERI (Mangin), p. 111.
A. CINNAMOMEUS Schiem., p. 164.
A. CITRISPORUS Von Höhnel, p. 191.
A. CLAVATUS Desm., p. 97.
A. CONICUS Blochwitz, p. 125.
A. disjunctus B. and S., p. 108.
A. ECHINULATUS (Delacr.), p. 107.
A. EFFUSUS Tiraboschi, p. 206.
A. erythrocephalus B. and C., p. 197.
A. FISCHERI Wehmer, p. 132.
A. FLAVIPES (B. and S.), p. 156.
A. FLAVUS Link, p. 199.
A. fontoynonti Guéguen, p. 115.
A. fumaricus Wehmer, p. 181.
A. FUMIGATUS Fres., p. 129.
A. fusco-cinereus Ellis and Morgan, p. 100.
A. GIGANTEUS Wehmer, p. 99.
A. GRACILIS Bainier, p. 125.
A. gratioti Sartory, p. 150.
A. HERBARIORUM SERIES MAJOR (Mangin), p. 106.
A. herbariorum series major var. violaceus (Mangin), p. 106.
A. HERBARIORUM SERIES MINOR (Mangin), p. 110.
A. INSUETUS (Bainier), p. 153.
A. JAPONICUS Saito, p. 171.
A. LUCHUENSIS Inui, p. 171.
A. LUTEO-NIGER (Lutz), p. 166.
A. MEDIUS Meissner, p. 108.
A. mutabilis B. and S., p. 110.
A. nanus Mont., p. 170.
A. NIDULANS (Eidam) Winter, p. 138.
A. NIGER Van Tieghem, p. 167.
A. OCHRACEUS Wilhelm, p. 188.
A. okazakii Okazaki, p. 157.
A. ORYZAE (Ahl.) Cohn, p. 198.
A. OSTIANUS Wehmer, p. 190.
A. PARASITICUS Speare, p. 203.
A. PENICILLOIDES Speg., p. 126.
A. penicillopsis (Hennings) Raciborski, p. 196.
A. PHOENICIS (Corda) Thom, p. 175.
A. pseudo-clavatus Purjewitsch, p. 100.
A. pulchellus (Speg.), p. 181.
A. PULVERULENTUS (McAlp.) Thom, p. 179.
A. QUERCINUS (Bainier), p. 186.
A. repandus B. and S., p. 109.
A. REPENS (Corda) Sacc., p. 113.
A. ruber (S. and B.), p. 112.
A. scheelei B. and S., p. 112.
A. SCHIEMANNI (Schiem.) Thom, p. 172.
A. sejunctus B. and S., p. 111.
A. SULPHUREUS (Fres.), p. 185.
A. SYDOWI (B. and S.), p. 147.
A. TAMARII Kita, p. 194.
A. TERREUS Thom, p. 150.
A. TERRICOLA Marchal, p. 192.
A. TERRICOLA var. AMERICANA Marchal, p. 192.
A. umbrosus B. and S., p. 110.
A. USTUS (Bainier), p. 152.
A. varians Wehmer, p. 127.
A. VERSICOLOR (Vuillemin) Tiraboschi, p. 142.
A. VIOLACEO-FUSCUS Gasp., p. 172.
A. WENTII Wehmer, p. 183.

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[1] Species names listed in small capitals are accepted by the authors for forms known to them in culture. Other species in the list are probably valid as representing forms which may be rediscovered and cultured.

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Sans Tache

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The Williams & Wilkins Company has the privilege of being publishers, in English, of Dr. F. d'Herelle's communications on the bacteriophage, or bacteria-destroying principle which was his own discovery.

The first book was in French. In 1922 this was revised and enlarged, and with the collaboration of Dr. George H. Smith was published in English under the title, *The Bacteriophage: Its Rôle in Immunity*. This work is now out of print.

In 1923 *Les Defenses de l'Organisme* was published. In 1924 this was in turn revised, enlarged and translated into English under the title *Immunity in Natural Infectious Disease*.

In 1926 a new work was published, incorporating some of the material of the 1922 book, but a much more extended treatise. This book *The Bacteriophage and Its Behavior* is material not hitherto published in any language; and in it Dr. d'Herelle reviews not only his own work but that of other investigators in bacteriophagy.

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